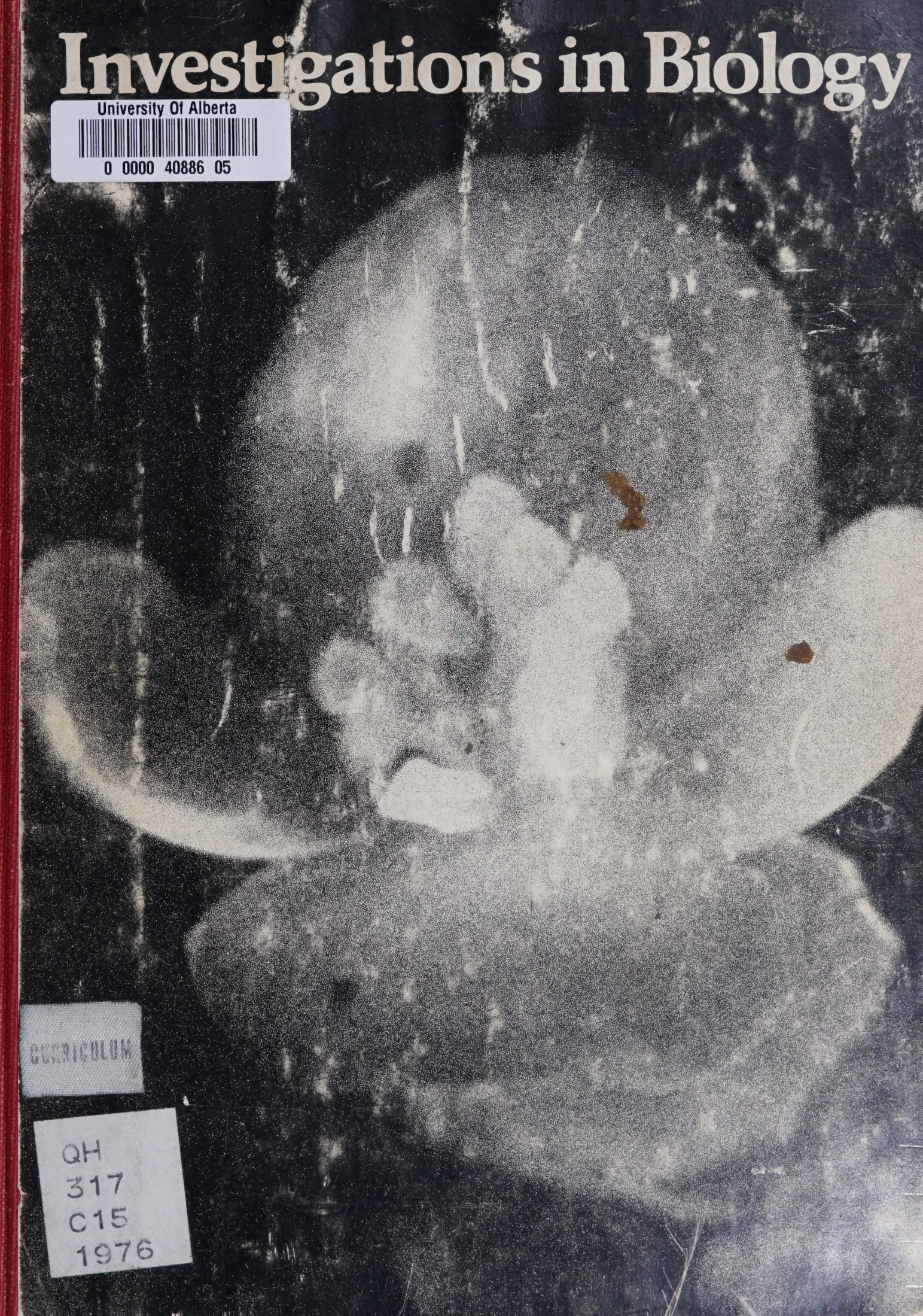
Investigations in Biology



Investigations in Biology

Prepared by:
THE CALGARY AND DISTRICT
BIOLOGY TEACHERS' ASSOCIATION

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Preface

The Calgary and District Biology Teachers' Association was approached to prepare a laboratory manual for the current Biology 30 curriculum. The association undertook the project and requested teachers, throughout the province, to submit exercises that they found useful in the teaching of the current biology course. From the exercises received and those developed by the five individuals who prepared the manual, this interim edition has resulted.

The intent of this edition is to provide a varied selection of activities that enhance the lecture material presented in the classroom. A variety of laboratory activities has been included in the manual. The areas covered include the following: 1) the chemical basis of life; 2) metabolism of cells; 3) digestion; 4) energy pathways; 5) photosynthesis; 6) gas exchange; 7) circulation; 8) excretion; 9) nervous coordination and 10) immunity. The authors felt that a choice of activities would provide flexibility for both the instructor and the student.

The preparation of this manual was the responsibility of Garth D. Benson, Eugene J. Shostal, Fred C. Hunt, David T. Lunn and Don A. Brookes. We are grateful for the contribution and advice of Ruth Benda, Dick Epton, Yvonne Helmer, Grace Lopez, Linda Raasveldt, Michael Robison, Carol Romine and Dave Severson.

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To the Students

This is a pilot edition of the Laboratory Manual which is being prepared to accompany the Biology 30 program. This means that the text as you read it is very much as it left the author's typewriter, before it has been edited into a final form.

By using this pilot edition, you are helping us to give the book its most severe test: is it a good book for you to learn from? You are contributing towards making this a better book for all the students who will eventually be using the finished text.

We want you to criticize the book. Be honest: tell your teacher exactly what you think of it, its good points and its bad points. What changes would you like to see made for the final version? Your teacher will be passing these comments on to us, as will the other classes in other schools who are taking part in this experiment. This is a unique opportunity for you to participate in the making of a good textbook.

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INVESTIGATION 1

IDENTIFICATION OF CARBOHYDRATES

INTRODUCTION:

Carbohydrates are molecules composed of carbon, hydrogen and oxygen atoms with the hydrogen and oxygen in the ratio of 2:1. Molecules of carbohydrates can range in size from a single glucose unit with a molecular weight of 180 to extremely large molecules with a molecular weight in excess of 500 000. The importance of carbohydrates is that they provide the source of energy for all organisms.

PURPOSE: To determine the types of carbohydrates that are found in various foods.

MATERIALS:

- 1% starch solution
- skim milk
- celery extract
- 1% glycogen solution
- potato extract
- 1% sucrose solution
- corn syrup
- carrot extract
- I_2-KI solution
- Benedict's solution
- 75% H_2SO_4
- cover slips
- microscope slides
- microscope
- 400 ml beaker
- Bunsen burner or hot plate
- test tubes to hold 10 ml
- test tube rack
- razor blade or scalpel
- drop plate

PROCEDURE: A) Reduced Sugars

Obtain a 10 ml sample of one food and place it in a clean test tube. To this extract add 5 drops of Benedict's solution. Heat the mixture in a hot water bath for 2-3 minutes and observe any color change. Repeat the procedure for each sample.

B) Starch

Place 5 drops of each sample in a drop plate and add 1 drop of iodine solution to each sample. Observe any color change other than the light brown color which is due to the iodine.

C) Cellulose

Mount sections or crushed fragments of celery, potato or carrot in the iodine solution. Observe the sample with the microscope and locate blue-stained starch. Place a drop of 75% H_2SO_4 at one side of the cover slip. As the acid diffuses in note the cellulose walls swell and become blue. (Caution: H_2SO_4 is corrosive).

RESULTS: Record your results in a chart similar to the one shown below.

SAMPLE	RESULT		OBSERVATIONS
	POS.	NEG.	
1.			
2.			
3.			

QUESTIONS:

1. What are the structural differences between the three classes of carbohydrates?
2. What is the structural difference between excess carbohydrates as they are stored in plants and animals?
3. Recent developments have indicated that athletes who eat a large amount of starchy food a few days prior to a contest have greater energy reserves than those who eat the "typical steak dinner" on the day of the game. Why should this be the case?
4. Explain why photosynthesis is necessary for the survival of most animals. Give an example of an organism that does not need photosynthesis for survival.
5. Explain why Benedict's solution and Fehling's solution only give you a positive test with reducing sugars.
6. Sucrose is a disaccharide which gives a negative test with Benedict's solution. Why?
7. Glucose and fructose are bonded together to produce a molecule of sucrose; how could you obtain a positive test for sucrose with Benedict's solution?

INVESTIGATION 2
FAT IDENTIFICATION

INTRODUCTION:

Fat, the chemical union of glycerol and fatty acids, is an essential molecular group within the body. It forms a portion of each cell membrane as well as acting as the major energy reserve within the body. To test for this compound use the following procedure.

PURPOSE: To identify lipids in common foodstuffs

MATERIALS: Any of peanuts, butter, meat products (hamburger etc.), milk; propanol, mortar and pestle, test tubes, brown paper.

PROCEDURE: A) Translucence

i. If the test item is solid place on a piece of brown unglazed paper and crush with pestle, or rub it into the paper.

ii. If the test item is liquid place a drop of the item on the brown unglazed paper.

iii. If plant or animal tissue is used, place the item in 15 ml of propanol and let this sit for 15 minutes. Place a drop of this solvent on the brown unglazed paper.

iv. A TRANSLUCENT SPOT INDICATES A POSITIVE TEST FOR THE LIPID.

B) The Emulsion Test

Place the test item in a mortar and add 15 ml of propanol. Crush the food and filter into a test tube. Add this to a second test tube containing equal amounts of water. Shake.

IF AN EMULSION IS FORMED THEN FAT (LIPID) IS PRESENT

C) Foods

To a series of foods (see previous list in laboratory manual) carry out the above tests indicating if fat is present (+) or absent (-).

RESULTS: THE FOLLOWING CHART MAY BE USED THROUGHOUT THIS ACTIVITY

F O O D	RESULT (+) OR (-)

QUESTIONS:

1. Which of the foods tested seemed to have the most obviously positive test results?
2. Which of the foods tested seemed to lack fat?
3. The chemical process forming fat is dehydration. Explain this process.
4. Enzymes working on the breakdown of fat are termed _____.
5. Explain where bile is formed and what its function is in fat breakdown.

INVESTIGATION 3

IDENTIFICATION OF PROTEINS

INTRODUCTION:

Proteins, in contrast to fats and carbohydrates, are complex molecules composed of carbon, hydrogen, oxygen and nitrogen atoms. Certain proteins may or may not have sulfur, phosphorus, or trace metals such as iron or copper.

PURPOSE: To identify the presence of proteins in various foods.

MATERIALS: 10% egg albumin

10% gelatin solution

blood plasma

test tubes

test tube rack

10 ml graduated cylinder

eye dropper

distilled water

Biuret test materials:

6M NaOH

0.5% $CuSO_4$

Xanthoproteic test materials:

Concentrated HNO_3

PROCEDURE: A) Biuret Test

Label the test tubes with the names of the solutions to be tested. Add 2 ml of each solution to the test tubes. Include a test tube with 2 ml of distilled water to act as a control. To each solution and the control add 2 ml of 6 M NaOH. Now add 4 drops of 0.5% $CuSO_4$ to the test tubes and gently shake the tubes to mix the solutions. Appearance of a purple color indicates the presence of proteins and specifically the presence of peptide bonds between amino acids.

B) Xanthoproteic Test

Label the test tubes with the names of the solutions to be tested and add 3 ml of each solution to the test tubes. A test tube with 3 ml of distilled water will serve as a control. To each solution and the control add 1 ml of concentrated HNO_3. Appearance of a yellow color indicates the presence of proteins and specifically the benzene nucleus.

RESULTS: Record your results in a chart similar to the one show in Exercise 1.

INVESTIGATION 4
VITAMIN C

INTRODUCTION:

Vitamin C can be tested for with indophenol which is a blue indicator that turns colorless in the presence of Vitamin C.

PURPOSE: To test foods for Vitamin C content.

MATERIALS: test tubes
test tube rack
hot plate
beaker
eye droppers
fruit juices (orange, lemon, grapefruit)
0.1% indophenol solution
10% ascorbic acid solution

PROCEDURE: Add 10 drops of indophenol solution to a test tube. Add drop by drop ascorbic acid, shaking the tube after each drop. Continue adding the ascorbic acid until the indophenol becomes colorless. Record the number of drops of ascorbic acid necessary to change the blue color of the indophenol colorless.

Add 10 drops of indophenol solution to a clean test tube and add drop by drop the fruit juice to be tested.

Record the number of drops required to obtain a color change.

Add a small sample of the fruit juice, to be tested, to a clean test tube.

Using a hot water bath, boil the sample for 1 minute.

Test the juice for Vitamin C as you did in the previous section.

(CAUTION: Test tubes should be clean and dry as water in the test tubes will affect your results.)

RESULTS:

SAMPLE	DROPS OF INDOL-PHENOL REQUIRED	NOT BOILED	BOILED
Ascorbic Acid			
Orange Juice			
Grape Fruit Juice			
Lemon Juice			
Rose hips			

QUESTIONS:

1. Devise an experiment that would give you an indication of the amount of Vitamin C in a fruit juice sample.
2. Why should foods containing Vitamin C not be exposed to the air?
3. What factors were included in your experiment that caused errors in your data?

QUESTIONS:

4. Why should you avoid using juices that have had "Vitamin C added"?

5. Which food had the greatest Vitamin C content?

INVESTIGATION 5

IDENTIFICATION OF AN UNKNOWN

INTRODUCTION:

Using the knowledge that you have obtained from the previous four lab activities you will be able to identify carbohydrates, fats, proteins, and Vitamin C in an unknown sample. Your instructor will provide you with an unknown and you will be marked on the number of substances that are correctly identified.

PURPOSE: To identify various foodstuffs in an unknown.

PROCEDURE: Obtain an unknown sample from your instructor and test it for carbohydrates, fats, proteins and Vitamin C. Record your results in a chart similar to the following.

RESULTS:

TEST	UNKNOWN NUMBER	NAME		
		RESULTS		OBSERVATIONS
		POSITIVE	NEGATIVE	
CARBOHYDRATES Reduced Sugars				
Starch				
Cellulose				
FATS Translucence				
Emulsion				
PROTEINS Biuret				
Xanthoproteic				
Vitamin C				

INVESTIGATION 6

ACIDS AND BASES

INTRODUCTION:

The acidity of solutions is very important to all life forms, thus the concept of acids and bases must be understood in order to investigate many of the reactions that occur in living systems. Acids play an important role in our daily lives; vinegar, dilute acetic acid, gives a flavourable tang to pickles and potato chips. Ascorbic acid, Vitamin C, found in citrus fruit, is considered a daily nutritional requisite. Acetyl Salicylic Acid, Aspirin is a chemical used by many individuals to remove the symptoms of many discomforts.

The acidity of a solution is a measure of the hydrogen ion concentration of the solution. Water forms a small, but definite amount of hydrogen ion in the following manner;

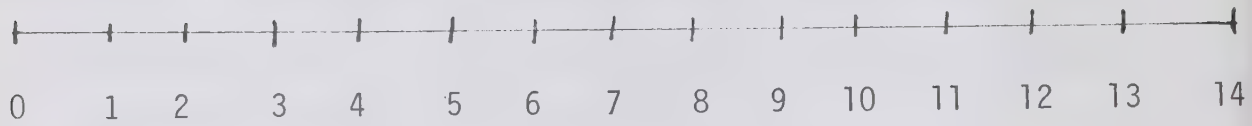
$$H_2O \rightleftharpoons H^+ + OH^-$$

Very few water molecules dissociate in this manner to form the ions indicated. In one litre of pure water there are 10^{-7} moles of hydrogen ions and an equal number of hydroxyl ions.

The pH unit is a useful means of expressing hydrogen ion concentration in physiological solutions. The pH is the exponent obtained when the concentration is expressed as a power of 10, with a change in sign of the exponent from negative to positive. For example, water has a pH of 7.

Molar H^+ conc.	H^+ conc.	pH
0.1	1×10^{-1}	1
0.01	1×10^{-2}	2
0.001	1×10^{-3}	3

pH Scale:



It can be seen that a solution of pH 3 is more acidic than a solution of pH 5. A solution of pH 3 is ten times more acidic, than a solution of pH 4 and 100 times more acidic than a solution of pH 5.

There are several means of determining the pH of solutions, some of which are more accurate than others. A more precise method involves the use of a pH meter, an instrument that is seldom readily available.

pH indicators are organic compounds that exhibit color changes at particular pH values.

Example:

INDICATOR	COLOR CHANGE	APPROXIMATE pH
methyl orange	red to yellow	3.5
congo red	blue to red	4.0
phenolphthalein	clear to red	9.7

These indicators are often combined and dried on strips of paper to provide a convenient means of estimating the pH of a solution.

PURPOSE: To determine the pH of various commercial products.

MATERIALS:

hand soap	milk
tooth paste	buttermilk
deodorant	orange juice
shampoo	lemon juice
oven cleaner	vinegar
tap water	pH paper or universal indicator solution

PROCEDURE: A) Experiment I

Use commercially prepared pH indicator paper or a solution of universal indicator, to estimate the pH of the following:

S A M P L E	pH
Hand soap	
Tap water	
Tooth paste	
Deodorant	
Shampoo	
Cleaning products (Oven cleaners)	

B) Experiment II

The sense of taste can be used to illustrate the exponential property of the pH scale. Hydrogen ions elicit a sour taste. Place a small amount of the materials to be tested in your mouth and roll them around on your tongue. At the end of each test rinse your mouth out with water. Do not attempt

B) Experiment II - Continued

to estimate the pH of the materials tested, instead compare the degree of "sourness" with tap water. When you have finished tasting and comparing the test materials then determine the pH of the materials with pH paper.

MATERIAL TO BE TASTED	COMPARISON WITH TAP WATER (HOW MANY TIMES MORE SOUR)	pH
Milk		
Buttermilk		
Orange juice		
Lemon juice		
Vinegar		

Relate your subjective taste experience to the actual pH of the materials. Lemon juice probably tests one or two pH units lower than orange juice. The taste (10 - 100 times more sour than water) serves as a reminder that the scale is indeed exponential.

QUESTIONS:

1. Explain why buttermilk tastes more sour than milk.

2. Botulism, a dangerous kind of food poison, is caused by the production of toxins in improperly processed canned food. The bacteria that produce these toxins cannot tolerate pH values below 7. List several canned foods that could act as a medium for this kind of bacterial growth.

3. Many industrial processes produce large amounts of oxides of nitrogen and sulfur. When these waste products are dumped into the atmosphere they combine with water vapour to produce nitrous and sulfurous acid. Precipitation, in areas surrounding these sites have a lowered pH level. Discuss the possible impact this practice may have upon surrounding ecological systems.

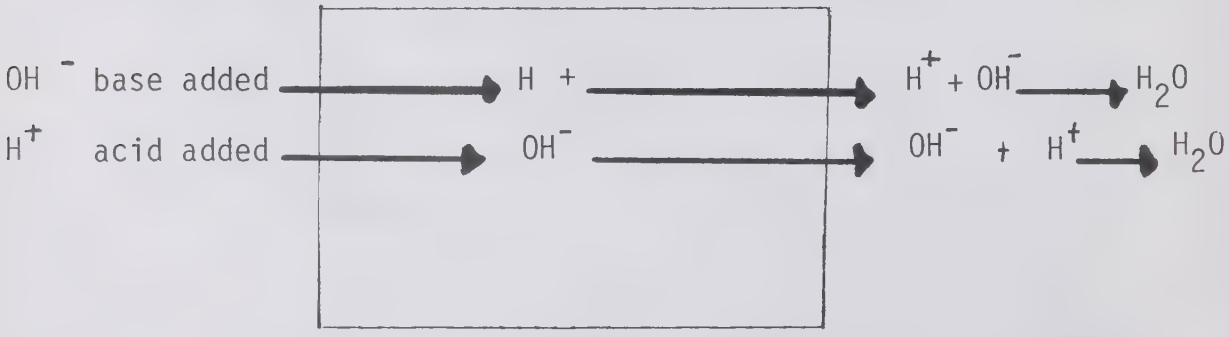
INVESTIGATION 7

BUFFERS

INTRODUCTION:

A buffer is a chemical system which tends to keep pH relatively constant. It contains a reservoir of both hydrogen and hydroxyl ions. When an acid is added to such a system the basic components are released to neutralize the hydroxyl ions. In this manner, the addition of acids and bases to a system are counterbalanced by the buffer system and therefore the pH is controlled.

BUFFER



Commercial antacids or buffers are sold to counteract the effects of hydrochloric acid concentration in gastric juice. Each of the companies that manufacture these compounds claim that product is more effective than those of their competitors.

PURPOSE: The purpose of this experiment is to compare the buffering action of several popular brands of antacids. You will also have an opportunity to observe buffer resistance to pH change when an acid is added.

MATERIALS: 100 ml graduated cylinder 0.1M HCl

250 ml Erlenmeyer flasks Burette

MATERIALS: (Continued)

200 ml beaker

eye dropper

methyl orange indicator

3 antacid tablets (each a different brand)

mortar and pestle

wax pencil

PROCEDURE: Place an antacid tablet in the mortar and grind to a fine powder. Use small amounts of water to transfer the powder from the mortar to the Erlenmeyer flask. Dilute to 50 ml and label "A". To this solution add 10 drops of methyl orange. Titrate the solution with 0.1 M HCl counting the number of drops required to sustain an end point (yellow to red) for 30 seconds. During the titration mix the solution by gently swirling the flask. A more accurate result can be obtained if a burette is used and the result is recorded in millilitres.

Repeat the procedure with brands "B" and "C".

RESULTS: Record your results in the following chart.

SAMPLE	GROUP RESULTS (ml of 0.1 M HCl)	CLASS RESULTS (ml of 0.1 M HCl)
Brand A		
Brand B		
Brand C		
Water		

QUESTIONS:

1. Name an organic compound, found in all cells which contains an organic acid group as well as organic base group.

2. Stomach contents must have a low pH (1 - 2) in order for protein digestion to occur. Discuss the problems that could arise from the continued use of antacids.

3. Which brand of antacid was most effective?

INVESTIGATION 8

EXTRACTION OF DNA AND RNA

INTRODUCTION:

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) each consist of a sugar, phosphate and nitrogenous groups arranged in a pattern. A model of DNA was first proposed by J. Watson and F. Crick. It was their work that established DNA as a double coiled helix. In this exercise you will be extracting both RNA and DNA from plants and spotting three of the four bases by means of paper chromatography.

PURPOSE: To extract nucleic acids from plant material and separate the bases of DNA and RNA by paper chromatography.

MATERIALS:

geranium leaves

clean fine sand

5% acetic acid

10% NaCl solution

0.5 M H_2SO_4

0.25 M $BaSO_4$

solvent of 15 parts acetic acid, 60 parts butanol and 25 parts of distilled water

mortar and pestle

hot plate

Whatman #1 filter paper

ultra-violet light source

centrifuge and centrifuge tubes

PROCEDURE: A) Wash the geranium leaves to remove any soil or foreign particles, add a small amount of sand and grind thoroughly for 5 minutes. Add 15 ml of 5% acetic acid to the plant material and grind for 3 minutes. Let the sand settle and decant the milky suspension into a centrifuge tube. Centrifuge this supernatant for 5 minutes to obtain solid material.

B) Save The Solid precipitate (nucleic acids and proteins) and discard the liquid portion that contains starch. The precipitate is stirred into 15 ml of 10% NaCl solution and it is then heated in a boiling water bath for 10 minutes. After heating, centrifuge for 3 minutes.

C) Save The Liquid supernatant from Part B by decanting it into another centrifuge tube and discard the solid coagulated proteins. Add to the centrifuge tube twice the volume of the supernatant that is already in the tube. Cool this mixture under cold running water, this will precipitate the nucleic acids. Centrifuge for 3 minutes.

D) Save The Solid precipitate and discard the liquid portion. Dissolve the solid nucleic acids in 1 ml of 0.5 M H_2SO_4 and heat for 30 to 60 minutes in a boiling hot water bath. (If the sample begins to dry out add sufficient distilled water to bring the sample back to the proper volume).

D) Continued

Neutralize the sample with drops of $Ba(OH)_2$. This will cause $BaSO_4$ to precipitate, ignore it. Stopper and label the test tube.

E) To separate the nitrogen-containing bases spot the nucleic acid mixture from 1 test tube on a strip of Whatman #1 filter paper. Use two spots and reapply five times allowing each spot to dry before each succeeding application. The two spots should be approximately 1.5 cm from the end of the paper and equidistant from each other.

F) Place the strip of filter paper into a test tube containing the solvent. The tip of the filter paper should be in the solvent, but the solvent should not be above the spot where the nucleic acids were applied. Place the test tube in a test tube rack and remove the paper when the solvent is within 1 cm of the top of the test tube.

G) Dry the filter paper and then expose it to ultra-violet light; the nucleic acids will appear as purple spots or bands. Mark these areas by drawing the outline of the fluorescent spots.

CAUTION Do not look into the U.V. light source, and avoid exposure to your skin for extended periods of time. U.V. light can cause eye and skin damage.

QUESTIONS:

1. What is the purpose of heating the mixture of NaCl, nucleic acids and proteins in part B?
2. How could you determine which nucleic acids have spotted out on the filter paper?
3. Structurally what is the difference between RNA and DNA?
4. Guanine is quite insoluble; how will this knowledge affect the expected outcome of this lab?
5. Explain the process involved in paper chromatography?

Teacher notes:

1. This lab involves waiting time ∴ approximately 2.5 hours are required to complete the lab.
2. The solutions can be kept in a refrigerator from one lab period to the next without apparent harm.
3. The precipitates will settle out if left undisturbed for a period of time, thus a centrifuge is not necessary. Extra care will be needed when decanting the supernatants if a centrifuge is not used.

INVESTIGATION 9

HOMEOSTASIS

INTRODUCTION:

The internal environment of an organism is in a state of dynamic equilibrium. Homeostasis is the term used to describe the maintenance of this state.

When homeostasis is considered the extracellular fluid that is found in an organism becomes very important. For example the human body is composed of water in which gases, minerals, foods, vitamins, hormones and wastes are dissolved. The water that is found in the human body may be divided into the fluid that is within the cell, the intracellular fluid, and that which bathes the outside of the cell, the extracellular fluid. The extracellular fluid is further subdivided according to its location. If it is found in blood vessels, it is called intravascular fluid, but if it surrounds the cells, it is called intercellular fluid. The constancy of the fluid in your body is controlled to a great degree by osmotic pressure.

Osmotic pressure of a solution can be estimated from the following equation:

$$\text{Osmotic pressure} = TcR$$

where T is the temperature in kelvins, c is the ionic concentration in moles per litre and R is a constant = 8.268 when the pressure is expressed in kilopascals.

PURPOSE: To develop an appreciation of the importance of homeostasis.

PROCEDURE: Answer the following set of questions.

1. What is homeostasis?
2. What part do body fluids play in homeostasis?
3. What is the relationship that exists between osmotic pressure and the concentration of the solute in a solution?
4. Explain how intracellular and extracellular fluids are affected when molecules such as glucose are taken into a cell. (In your answer refer to osmotic pressure).
5. Why does a substance that dissociates produce a higher osmotic pressure than one that does not dissociate?
6. Calculate the osmotic pressure of your blood that results from the glucose that is found in it. The average concentration is 80 - 120 milligrams per 100 millilitres of blood.
7. What is the concentration of a 0.9 M NaCl solution? (Molecular weight - 58.45.)
8. Explain the control that is used to regulate the glucose level in your blood.
9. What type of feedback system is operating in question 8?
10. Use the opposite feedback to your answer of question 9 and discuss the effect this type of feedback would have on homeostasis.
11. Explain how a decrease in the production of parathyroid hormone affects the level of Ca^{2+} found in the extracellular fluid, the permeability of the intestines and the storage of calcium in the bones.

INVESTIGATION 10

DIFFUSION THROUGH SEMI-PERMEABLE MEMBRANES

INTRODUCTION:

Diffusion is the movement of molecules or ions, due to Brownian motion, from an area of high concentration to an area of low concentration.

PURPOSE: To demonstrate that molecules diffuse through semipermeable membranes at various rates. The human digestive tract readily exhibits this and these conditions will be simulated in this activity.

MATERIALS:

- dialysis tubing
- 5% glucose solution
- 5% starch solution
- vegetable oil
- 5% NaCl
- 5% $AgNO_3$
- 5% gelatin solution
- string
- Benedict's solution
- iodine solution
- biuret solution
- test tubes to hold 10 ml
- 250 ml beakers
- 100 ml beaker
- brown unglazed paper

PROCEDURE: Cut a 15 cm strip of dialysis tubing and open to form a tube. Tie one end with string and half fill this sack with equal amounts of prepared glucose, starch, gelatin, oil and salt solutions. Tie off the other end of the sack, test for water tightness, wash the sack, fold it in half and place in the 100 ml beaker such that the tied ends are up out of the beaker. Fill this beaker with water that has been warmed to 37°C. Let this stand for 5 minutes then run tests on the water in the 100 ml beaker to see if any or

all of the chemical groups have passed through the membrane. TESTS FOR SUGARS, STARCH, PROTEIN AND FATS MAY BE FOUND IN PREVIOUS ACTIVITIES. Replace any water used from the 100 ml beaker for these tests with water heated to 37°C.

Test for Chloride (Cl^-) Add a few drops of silver nitrate solution to a water sample. A white precipitate indicates the presence of chloride ions.

OBSERVATIONS: The following chart may be used to record your observations:

MOLECULAR GROUP	TIME (+INDICATES PRESENT) (-INDICATES ABSENT)					
	5 min	10 min	15 min	20 min	25 min	30 min
Glucose						
Starch						
Fat						
NaCl						
Gelatin						

CONCLUSION: Make a statement indicating what you have learned about the movement of molecules through selectively permeable membranes

QUESTIONS:

1. What do you think would have been the change (if any) in the results if cold water had been used in place of the warm water?
2. Tyrosine is an amino acid. Would you expect to have a positive test for it in this activity? Explain your answer.
3. In a cell, turgidity is due to internal pressure. Describe the state of turgidity of the dialysis sack over the duration of the experiment. If there were changes suggest why these changes occur.
4. Define the terms hypotonic, hypertonic and isotonic.
5. Based on this experiment, suggest a "quick" energy food that you as a human could utilize.
6. Account for the difference in the rate of diffusion of the molecules.

INVESTIGATION 11

DIFFUSION

PURPOSE: To measure the influence of concentration on rate of diffusion.

MATERIALS:

potato	250 ml beakers
10% Lugol's iodine	razor blade
1% Lugol's iodine	ruler
0.1% Lugol's iodine	graph paper

PROCEDURE: Cut the potato into fifteen 1 cm cubes. Prepare three concentrations of Lugol's solution - by volume 10% (10 ml of Lugol's to 90 ml distilled or tap water), 1% (1 ml to 99 ml) and 0.1%(0.1 ml to 99.9 ml). Place these three solution concentrations in separate, labelled beakers. Into each beaker place 5 pieces of potato. At 5 minute intervals remove a potato cube from each solution and cut it open with a razor blade. Measure the distance the Lugol's solution has diffused into the potato cube.

RESULTS:

TIME IN SOLUTION (MINUTES)	DISTANCE OF DIFFUSION (mm)		
	10% con.	1% con.	0.1% con.
5			
10			
15			
20			
25			

Graph your results - x axis for time (minutes) and the Y axis for distance (mm).

CONCLUSION: What affect does the concentration of the iodine solution have on the rate of diffusion?

INVESTIGATION 12

ACTIVE TRANSPORT IN LIVING CELLS

INTRODUCTION:

At one time it was believed that the movement of molecules in and out of living cells could be attributed to the process of diffusion and osmosis. It is now known that living cells can accumulate and force out various molecules against a concentration gradient, opposing the processes of diffusion and osmosis. For example, it is known that some varieties of algae can accumulate iodine and certain primitive chordates, the tunicates, can accumulate vanadium. In both examples they accumulate approximately 2 000 000 times the concentration of the ion found in sea water.

This pumping of water or solute molecules in or out of, against a concentration gradient, requires the expenditure of energy. A cell is able to move molecules against a gradient only as long as it is carrying on metabolic activities. When a cell is treated with a metabolic poison, such as cyanide, it loses its ability to produce energy and hence it also loses its ability to maintain a concentration gradient.

In conclusion, active transport can be defined as an energy-requiring process which involves the movement of chemical molecules through a differentially permeable membrane against a diffusion gradient. The end result of this process yields an abundance of certain molecules on one side of a semipermeable membrane and a lack of the same molecules on the opposite side of the membrane.

PURPOSE: To study active transport in living cells.

MATERIALS: test tubes and test tube holder

2% Congo Red solution

1% yeast suspension

microscope

microscope slides and cover slips

Bunsen burner or hot plate

eye droppers

beakers

ring stand and ring

PROCEDURE: Label two test tubes and add 5 ml of living yeast suspension to each test tube. Place one test tube in a hot water bath and boil the contents for 10 minutes. Cool the test tube and add 6 drops of 2% Congo Red to each test tube. Gently shake the contents of each tube to mix the dye and the yeast suspension. Place a drop of the mixture from each test tube on appropriately labelled slides and observe under the microscope. (Note - use different eye droppers when transferring each mixture to the slides). Examine the slides under low, medium and high power and record your observations in a suitable chart. You should pay particular attention to the distribution of the dye.

QUESTIONS:

1. What is the effect of heat on living yeast cells?
2. Did you find any yeast cells that did not contain dye? If so explain.

QUESTIONS: Continued

3. Explain your observations of the heated solution.

4. Give two advantages of a living cell having the capacity to move molecules at will.

INVESTIGATION 13

SOL-GEL PHASE REVERSALS

INTRODUCTION:

Protoplasmic properties depend on the physical state of the material as well as the types and quantities of substances present. When various molecules are mixed with water a number of physical states can exist. These states are differentiated by the size of the dispersed molecules. These states are:

1) True solutions - consist of ions or molecules dispersed among the solvent. The solute particles are very small (0.0001 μm or less in diameter) and they very rarely settle out.

2) Colloids or Colloidal Suspensions - consist of solute particles of intermediate size (0.1 to 0.0001 μm in diameter) dispersed in water. The particles of a colloidal solution have the same type of electrical charge and therefore tend to repel each other. This repulsion keeps the particles dispersed and suspended.

3) True suspensions - consist of particles that are large (0.1 μm or larger). These particles tend to settle out if they are allowed to stand for a period of time.

In terms of biology, colloids are unique because they have the ability to undergo phase changes. These phase changes may occur because of changes in:

a) temperature

b) pH

c) ions

d) the amount of water present

e) the concentration of solute molecules and

f) pressure

Protoplasm is a colloidal system with protein molecules and water forming the two phases. The normal functions of protoplasm depend upon the rapid change from one phase to another. Cellular colloids undergo sol-gel transformations constantly and if protoplasm is converted irreversibily to a sol or a gel, the cell dies.

PURPOSE: To study the physical and chemical factors on sol-gel transformations. (Note: this investigation would be best done as a demonstration.)

MATERIALS: test tubes and test tube rack

10% gelatin solution

distilled water

Bunsen burner or hot plate

ring stand and ring

beakers

NaCl (crystalline)

$CaCl_2$(crystalline)

$CaSO_4$(crystalline)

grease pencil

PROCEDURE: Label five test tubes and add 10 ml of distilled water and 1 g of powdered gelatin to each test tube. To dissolve the gelatin heat the test tubes in warm water. Cool all five test tubes by suspending in cold water or ice. Observe and explain the results.

a) Effect of Temperature

Heat test tubes 1 and 2 in a hot water bath until a change of state occurs. DO NOT allow the contents of the test tubes to boil. Observe what happens to the contents of the test tubes.

b) Effect of Dilution

Add an additional 20 ml of distilled water to test tube number 1 and gently shake. Cool the contents of tubes 1 and 2. Explain what happens to the colloidal solution in each test tube.

c) Effect of Ion Concentration

Add 1 g of crystalline $CaSO_4$ to test tube number 3;
1 g of crystalline NaCl to test tube number 4;
and 1 g of crystalline $CaCl_2$ to test tube number 5.
Set the three test tubes aside for 24 hours and observe the contents the next day. Explain what happens.

QUESTIONS:

1. Suppose you were a protein molecule in a colloid; how would you describe your position in relation to other solute and solvent particles in a sol and a gel?

INVESTIGATION 14

ENZYMES

INTRODUCTION:

Hydrogen peroxide is formed in living cells as a by-product of metabolism. It acts as a poison and cells must break it down immediately or be destroyed. An enzyme, catalase, is required to decompose the hydrogen peroxide. The enzyme is found in most cells such as fresh or frozen liver, spinach or potatoes.

PURPOSE: To compare the action of catalase to a non-protein catalyst and examine the action of a catalyst under different conditions.

MATERIALS: test tubes and test tube rack

3% H_2O_2

clean sand

manganese dioxide

fresh or frozen liver

fresh potato

stirring rod

Bunsen burner or hot plate

beaker

ice

PROCEDURE: a) Catalytic Reactions

Add 2 ml hydrogen peroxide (H_2O_2) to two test tubes. Place 0.1 g of fine sand in one test tube and to the second test tube add 0.1 g of manganese dioxide.

PROCEDURE: (Continued)

A) Observe and record the rates of reaction. What is the gas that is bubbling off?

B) The effect of an enzyme

Add 2 ml of hydrogen peroxide to two clean test tubes. Place a piece of liver about the size of a rice grain into one and a piece of potato into the other. Record the rates of reaction and compare the liver and potato results to manganese dioxide.

C) Re-using an enzyme

Divide the liquid portion of the liver tube into two clean test tubes. Cut the piece of liver from the original liver test tube into two equal portions and place these in the two new test tubes. To the first test tube add a fresh piece of liver, and to the second test tube add 1 ml of hydrogen peroxide. Record your observations. Explain the reaction with the piece of new liver. What would happen if you added additional H_2O_2 to the test tube with the old liver?

D) Effect of particle size

Place a small piece of liver and potato in two clean test tubes and add a pinch of sand to each. Crush the materials with a stirring rod. Add 2 ml of H_2O_2 to each test tube. Compare the results with uncrushed liver and potato from Section B. Observe the speed of the reactions.

E) Effect of temperature

Place a small piece of liver in a test tube and heat it for 5 minutes in a boiling water bath. Add 2 ml of fresh H_2O_2 to the boiled liver and record the results. Place 2 ml of H_2O_2 in two clean test tubes, place one test tube in a 37°C hot water bath for 5 minutes and the second test tube in an ice water bath for the same length of time. Remove both test tubes from the water baths and add a small piece of liver. Compare the rates of reaction.

RESULTS: Use the following chart to record the rates of reactions: 0 - no reaction, 1 - slow, 2 - moderate, 3 - fast, and 4 - extremely rapid.

TEST	RATE OF REACTION					OBSERVATIONS
	0	1	2	3	4	
Manganese dioxide						
Sand						
Piece of liver						
Piece of Potato						
Old liver and new liver						
Old liver and new H_2O_2						
Crushed liver						
Crushed potato						
Boiled liver						
Boiled H_2O_2						
H_2O_2 in ice						

QUESTIONS:

1. Compare the ratings of the reactions in sections A, B, C, D, and E of the procedure.
2. How do you account for the differences in the rates?
3. Can H_2O_2 be broken down by catalysts other than those found in living systems? Explain your answer.
4. Describe the effect of temperature and particle size on the rate of enzyme action.
5. The body temperature of a dog is approximately $40^{o}C$. How would pieces of a dog's liver affect your results in this experiment?
6. At what temperature would the reaction occur most rapidly if you used pieces of dog liver?
7. The human stomach has a pH of about 2. What effect would this pH have on catalase? Suggest a test to determine if your answer is correct.

INVESTIGATION 15

THE EFFECT OF TEMPERATURE ON ENZYME ACTIVITY

INTRODUCTION:

Enzymes are large complex protein molecules hence temperature will have a dramatic effect on them. High temperatures tend to denature enzymes and this prevents any further reactions from taking place. Extremely low temperatures generally do not destroy enzymes but they reduce the level of activity to almost zero. These two states lead you to conclude that enzymes have an optimum temperature at which they work best.

PURPOSE: To demonstrate the affect of varying temperatures on the enzymatic hydrolysis of starch.

MATERIALS: water baths set at 10°C, 20°C, 37°C, and 40°C
beakers
test tubes and test tube rack
Bunsen burner or hot plates
ring stands
wire gauze
thermometers
graduated cylinders
1% starch solution
dilute iodine solution
saliva
cheesecloth to strain saliva
ice

PROCEDURE: Prepare the water baths at the temperatures indicated above. To each of 4 test tubes add 5 ml of 1% starch solution and 1 ml of dilute iodine. Mix thoroughly and place one tube in each water bath. Add 4 ml of saliva to another 4 test tubes and place in the water baths. Allow a few minutes for the temperature of the solutions and saliva to reach that of the bath.

Check periodically to see that the temperatures remain constant. Heat gently or add more ice as required. When the solutions and saliva are at the desired temperatures, combine by adding the saliva to the starch-iodine solution. Record the time required for the disappearance of the blue-black color in a chart format. Interpret the results by explaining what happened in each tube.

QUESTIONS:

1. How would a control be prepared for this investigation?

INVESTIGATION 16

THE INFLUENCE OF pH ON ENZYME ACTIVITY

INTRODUCTION:

Enzymes are sensitive to pH. These protein molecules work most effectively at a specific pH. For example pepsin works most effectively at a pH of 1-2 while trypsin is most effective at pH 8.

PURPOSE: To study the role pH plays in enzyme activity.

MATERIALS: 5 test tubes

1% starch solution

eye droppers

spot plates

iodine solution

buffer solutions of pH 3, 5, 7, 9

saliva

PROCEDURE: Chew on an elastic band to help salivation and collect this saliva in a clean test tube until it is ¼ to ½ full. Add an equal amount of tap water. Filtration through cheesecloth may be required at this time to remove the "head". Into four test tubes place 2 ml of the starch solution and 2 ml of the pH solutions. Label each test tube according to the pH of the solution it contains, and mix them thoroughly. To each test tube add 1 ml of the saliva solution and mix this in thoroughly.

PROCEDURE: Continued

At one minute intervals the presence of starch may be tested for by removing a drop from each tube, placing it in the spot plate and adding iodine. Should starch be present the iodine will stain this complex blue/black.

As digestion occurs this blue/black color will give way to a red color (dextrins present) and finally no color will appear other than the iodine.

OBSERVATIONS: The following chart may be used to record your observations. Indicate color noted beside each time and under the appropriate pH.

TIME (minutes)	buffer pH			
	3	5	7	9
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				

CONCLUSION: Make a statement in reference to the purpose of this activity.

QUESTIONS:

1. At what pH is saliva most active?
2. A pH of 9 would most closely simulate conditions in what area of the digestive tract?
3. Which test tube used would have a pH that would simulate conditions in the small intestine?
4. Pepsin is a proteinase secreted by the stomach. Discuss the pH range that this enzyme is active in and suggest what likely happens to this enzyme structurally as it leaves the stomach?
5. Digestion is exemplified by one specific process. This process is hydrolysis. Briefly explain what hydrolysis is using a molecule of fat as an example.
6. Briefly explain the role of enzymes in digestion.

INVESTIGATION 17

RATE OF WATER TRANSPORT IN PLANTS

(RECOMMENDED AS A DEMONSTRATION)

PURPOSE: To study the influence of leaves on the rate of water uptake in a plant.

MATERIALS: 2 stalks of celery
safranin (dilute)
beakers
ruler

PROCEDURE: Place 100 ml of safranin red dye in each beaker. To the first beaker add a freshly cut stalk of celery. To the second beaker add the second stalk of celery which has been freshly cut and the leaves have been removed. Over the period assign a recorder to keep a record of the movement of the dye upward in the xylem of the stalks.

RESULTS: Record the movement of the dye in the following chart.

TIME (MINUTES)	RATE OF MOVEMENT (mm)	
	WITH LEAVES	WITHOUT LEAVES
10		
20		
30		
40		
50		

QUESTIONS:

1. What effect do the leaves have on the movement of water in the xylem?

INVESTIGATION 18

THE ABSORPTION OF WATER BY CELLS

INTRODUCTION:

When cells are placed in a hypotonic solution water moves into the cell by the process of osmosis. In some animal cells this process continues until the cell membrane swells and ruptures. In the case of blood cells this is known as haemolysis.

In plant cells, the membrane swells until it reaches the elastic limit of the surrounding cell wall. At this point the plant cell is said to be turgid.

PURPOSE: To observe the affects of osmosis in red blood cells and pea seeds.

MATERIALS:
- tooth picks
- concavity slides
- microscope
- sterile blood lancets
- cotton
- ethanol
- distilled water
- 2% NaCl solution
- 0.9% NaCl solution
- glass jar with a tight fitting lid
- pea seeds
- sand
- battery jar

PROCEDURE: A) Haemolysis

Obtain three concavity slides and label them as follows: distilled water, 0.9% NaCl, and 2% NaCl. Sterilize your finger with the ethanol and prick the finger with a sterile lancet. CAUTION - do not exchange blood lancets with other students. Add one drop of blood to each slide and then add 1 drop of the appropriate solution to the slides. Stir the mixture with a clean toothpick and observe the three slides under the microscope. Observe the slides immediately and then every three minutes for fifteen minutes.

B) Imbibition

Obtain a glass jar with a tight fitting lid and add sufficient pea seeds to fill the jar. Add sand to the jar to fill the spaces that occur between the seeds. Cover the sand and seeds with water and place the lid securely on the jar. Place the jar containing the sand and seeds in a battery jar and cover it with water. Observe the jar over the next few hours and at 24 hours.

INVESTIGATION 19

ACTION OF GUARD CELLS

INTRODUCTION:

The specialized structure of guard cells allows them to regulate the size of stomatal openings. The inner walls bordering the stomatal openings are thick and heavy and therefore are less elastic than the outer walls. When guard cells are turgid they are open and when flaccid they are closed.

PURPOSE: To examine the mechanism of guard cell action.

MATERIALS: lettuce, purple heart and geranium leaves

0.5 M NaCl solution

eye dropper

slides

cover slips

microscope

Petri dish

distilled water

forceps

PROCEDURE: Place a leaf of fresh lettuce in a Petri dish. Add sufficient distilled water to half fill the dish and let the leaf stand for 5 minutes until it is turgid. Cut a small piece of the leaf. Bend this section of the leaf so that the epidermis is intact but the remainder of the leaf has broken. Pull a piece of the epidermis from the leaf and mount it on a slide with a drop of distilled water. Locate

PROCEDURE: Continued

a stomatal opening and the surrounding guard cells. Observe the state of the guard cells and describe two differences between guard cells and epidermal cells.

Add a drop of 0.5 M NaCl solution to the edge of the cover slip and draw the saline under the cover slip by means of a paper towel. Observe and describe the stomatal opening.

Add two drops of distilled water to flush the NaCl from the cells. Observe and record the effect the water has on stomatal openings. The last two steps may have to be repeated several times to observe any changes.

Count the number of stomatal openings in the medium power field of view. Prepare a wet mount of the other epidermal surface and count the number of stomata with the medium power.

Count the number of stomata on both epidermal surfaces of the purple heart and geranium leaves.

RESULTS: Record your observations in the following chart.

SAMPLE	DISTILLED WATER	0.5 M NaCl	NUMBER OF STOMATA		OBSERVATIONS
			UPPER EPIDERMIS	LOWER EPIDERMIS	
LETTUCE					
PURPLE HEART					
GERANIUM					

QUESTIONS:

1. What is the mechanism by which guard cells regulate stomatal openings?
2. What effect would covering the upper epidermis with petroleum jelly have? The bottom? Both surfaces?
3. Suggest three environmental factors that will affect guard cell activity.
4. Why do some plants reduce their rate of photosynthesis during the heat of midday?
5. Account for the difference in the number of stomatal openings from the upper and lower epidermis of a plant.
6. Why do different plants have different numbers of stomatal openings?

INVESTIGATION 20

TURGOR PRESSURE

INTRODUCTION:

Turgor is the state of a cell in which the cell wall is rigid. The increase in the volume of the vacuole and protoplasm causes the cell wall to stretch. Osmosis is responsible for the movement of water into the cell thus creating a cell which is turgid.

PURPOSE: To demonstrate turgidity in plant cells.

MATERIALS: peeled potato
razor blades
centimetre ruler
0.125 M, 0.25 M, 0.5 M and 1.0 M sucrose solutions
beakers

PROCEDURE: Prepare four pieces of potato each 4 x 0.5 x 0.5 centimetres. Accurately measure each piece of potato and record the results in the data chart. Place 25 ml of each sucrose solution in four beakers. Place one piece of potato into each beaker. Measure each piece of potato after 24 hours and record the measurements in the data chart.

RESULTS:

CONCENTRATION OF SUCROSE	SIZE OF POTATO RECTANGLE BEFORE SOAKING	SIZE OF POTATO RECTANGLE AFTER 24 HOURS	CHANGE IN VOLUME
0.125 M			
0.25 M			
0.5 M			
1.0 M			

QUESTIONS:

1. Why does a plant wilt when it does not receive sufficient water?
2. What will the osmotic pressure be in a plant cell when it is in an isotonic solution?
3. Explain plasmolysis in plants.
4. What will occur when a potato rectangle is placed in a hypotonic solution? Explain your answer.
5. Using the following information calculate the osmotic pressure of the potato sample in the 0.5 M solution.

   Osmotic pressure = $\frac{2270 \times \Delta}{1.86}$ kPa

   where Δ is observed freezing point depression in degrees Celsius of the unknown solution. A 1 M solution of an unionized substance has a measured freezing point depression of 1.86°C compared to pure water.

INVESTIGATION 21

INORGANIC IONS IN PROTOPLASM

INTRODUCTION:

There are a number of ions that are commonly found in living tissues. Some of these ions can be tested for in a qualitative manner using a minimum of equipment. The following tests can be used to identify the presence of some of the biologically essential inorganic ions.

PURPOSE: To identify some of the inorganic ions found in living plant tissue.

MATERIALS:
- grease pencil
- test tubes
- test tube rack
- distilled water
- 3 M ammonium thiocyanate solution
- 3 M HCl
- 0.5 M ammonium molybdate (as solutions A and B)
- metallic tin
- 0.25 M ammonium oxalate solution
- 0.08 M sodium cobaltinitrite solution
- 95% ethanol
- 0.25 M barium chloride solution
- 1 M sodium hydroxide solution
- 0.15% Clayton (Titan) yellow solution
- 0.5 M silver nitrate solution
- plant filtrate

NOTE: Consult the instructor's notes at the conclusion of this exercise concerning the above solutions.

PROCEDURE: Label eight clean test tubes and add 2 ml of the filtrate supplied by your instructor to each test tube. Use test tube number 1 as a control to make a comparison in one of the ion tests.

A) Iron (Fe^{3+})

Add 6 drops of 3 M ammonium thiocyanate solution and 2 ml of 3 M HCl to the filtrate in test tube number 2. Gently shake the mixture; a faint pink color indicates the presence of iron.

To make a comparison, add 2 ml of 3 M HCl and 6 drops of ammonium thiocyanate to the control. Hold the two test tubes side by side and compare the colors of the solutions. A white sheet of paper used as a background will make your observations somewhat easier.

B) Phosphate (PO_4^{3-})

To the filtrate in test tube number 3 add 10 drops of freshly prepared 0.5 M ammonium molybdate solution. It is imperative that this reagent be mixed immediately prior to use. The reagent is prepared by adding one part of solution A to 2 parts solution B. Shake this mixture well. After the ammonium molybdate solution has been added to the filtrate add a small piece of metallic tin to the test tube. Do not agitate the contents in any way. The formation of a blue color around the tin indicates the presence of the phosphate radical.

PROCEDURE: Continued

C) Calcium (Ca^{2+})

Add 6 drops of 0.25 M ammonium oxalate to the filtrate in test tube number 4. Shake the contents of the test tube. The presence of calcium ions is indicated by the formation of a fine white precipitate.

D) Potassium (K^{+})

To the filtrate in test tube number 5, add 6 drops of 0.08 M sodium cobaltinitrite solution. This solution must be freshly prepared. Gently shake the contents of the test tube and add 2 ml of 95% ethanol. If potassium ions are present a yellow precipitate will form.

E) Sulphate (SO_4^{2-})

Add 3 drops of 0.25 M barium chloride to the filtrate in test tube number 6. If a white precipitate forms the sulphate radical is most likely present.

F) Magnesium (Mg^{2+})

The filtrate in test tube number 7 should be made slightly basic with the addition of 3 to 4 drops of 1 M sodium hydroxide. This can be checked by using litmus paper. If the solution is basic gently shake the contents of the test tube and then add 2 drops of 0.15 % Clayton yellow. The Clayton yellow must also be freshly prepared. The magnesium ion is indicated by colors ranging from deep orange to pink.

PROCEDURE: Continued

G) Chloride (Cl^-)

To the filtrate in test tube number 8, add 2 drops of silver nitrate. A white precipitate indicates the presence of chloride ions.

Repeat each test using distilled water and tap water.

RESULTS: Record your results in the following chart.

I O N	PLANT SAMPLE	DISTILLED WATER	TAP WATER	O B S E R V A T I O N S
Phosphate				
Calcium				
Potassium				
Sulphate				
Magnesium				
Chloride				
Iron				

QUESTIONS:

1. What is the major source of the minerals that are found in plants?
2. What process is used by the plant in accumulating these ions?
3. At what point on the plant are these ions absorbed? Why is it restricted to a small area?

Solutions used in Investigation 21.

Ethanol 75%

95% ethanol 79 ml

distilled water - add to total 100 ml

Ammonium Molybdate 0.5 M

This solution should be prepared in two parts; the two solutions are to be mixed immediately prior to use.

Solution A

15 M ammonium hydroxide	16 ml
ammonium molybdate	20 g
distilled water	80 ml

Add the ammonium hydroxide to the distilled water. Dissolve the ammonium molybdate in this solution. Filter or decant this solution and store the filtrate in a tightly stoppered bottle.

Solution B

16 M nitric acid	80 ml
distilled water	120 ml

Mix this solution thoroughly. Caution: HNO_3 is very corrosive, follow standard safety procedures.

For use during the phosphate test add one part solution A to 2 parts solution B and stir vigorously.

Ammonium oxalate 0.25 M

ammonium oxalate 3.5 g

distilled water add to total 100 ml

Ammonium thiocyanate 3 M

ammonium thiocyanate 22.8 g

distilled water add to total 100 ml

CLayton (Titan) Yellow 0.15%

Clayton yellow 0.15 g

75% ethanol 100 ml

Dissolve the dye in the ethanol and keep the resulting mixture in a tightly stoppered container.

Silver Nitrate 0.5 M

silver nitrate 8.5 g

distilled water add to total 100 ml

Store in a tightly stoppered brown bottle.

Sodium Cobaltinitrite 0.08 M

This solution must be freshly prepared due to its instability.

Cobaltous nitrate $Co(NO_3)_2 \cdot 6H_2O$ 2.5 g

Glacial acetic acid 2 ml

Sodium nitrite 25 g

distilled water add to total 100 ml

Dissolve the sodium nitrite in 75 ml of distilled water. To this solution add the 2 ml of glacial acetic acid. Dissolve the cobaltous nitrate in the acidified solution and allow this mixture to stand for one day.

Filter the resulting solution and dilute the filtrate with distilled water to make up a 100 ml quantity.

Sodium Hydroxide 1 M

sodium hydroxide	4 g
distilled water	add to total 100 ml

Plant Filtrate

Dry some plant material, preferably leaves, in an oven set at low heat for 24 hours. Other methods such as plant presses and then air drying the resulting material could also be used. Ignite the dried plant material in a crucible. Bunsen burners, kilns or a self cleaning oven can be used to reduce the plant material to inorganic ash. If a small amount of carbon remains it will not harm the results. Dissolve the resulting ash in 15 ml of 2.5% acetic acid. Filter this mixture and store in a stoppered container for future use. If large amounts of filtrate are required the white ash from a fireplace may be substituted. A corresponding increase in acetic acid will also have to be used. Fireplace ash is not recommended unless the ash is of one species and it is not contaminated.

INVESTIGATION 22

PHOTOSYNTHESIS

INTRODUCTION:

Photosynthesis involves a series of chemical reactions whereby light energy is converted into chemical energy. This chemical energy is stored in the form of bond energy within organic compounds. Photosynthesis, therefore, is a chemically constructive process.

It has been found that one of the chief end products of photosynthesis is a carbohydrate. Organisms containing chlorophyll are capable of carrying out a synthesis reaction where carbohydrates are produced. Plants store these end products as monosaccharides, disaccharides or polysaccharides. In Exercise 1 the test for reduced sugars was discussed and the same procedure will be followed in this exercise to determine if light is necessary for photosynthesis to occur.

PURPOSE: To demonstrate that light is necessary for photosynthesis to occur.

MATERIALS:

test tubes	Bunsen burner or hot plate	
test tube rack	scissors	
mortar and pestle	centrifuge and tubes	
fine, clean sand	eye droppers	
filter paper	marking pencil	
funnel	Benedict's solution	
ring stand	10% glucose solution	
iron ring	distilled water	
beaker	Testape	2 plants

PROCEDURE: Note - prior to this exercise suitable green plants such as beans, peas, Coleus or Zebrina should be cultivated. If bean plants are used, the two leaf stage is ideal for this experiment.

Place one plant in the dark for 72 hours while the other plant remains in the light during this time period. Mark both plants, indicating the dark and light condition. After the time period has passed, remove the plants from their respective locations and carry out the following procedure. It is most important that contamination of the plant extracts or mixing of materials does not occur.

Remove 2 leaves from the plant kept in the dark. Cut the leaves into small pieces and place them in a mortar. Add 10 ml of distilled water and a very small pinch of sand, grind this mixture thoroughly. Filter the mixture and centrifuge the filtrate for 5 minutes. Decant the liquid (supernatant) and discard any precipitate. Store the liquid extract in a clean test tube marked Dark Filtrate. Repeat this procedure for the plant exposed to the light. The second filtrate should be stored in a clean test tube marked Light Filtrate.

Mark five clean test tubes in the following manner: BEFORE, DARK, LIGHT, GLUCOSE, DISTILLED WATER.

To each of these five test tubes add 2 ml of Benedict's solution. Using clean eye droppers add 10 drops of the dark filtrate to the DARK test tube. To the test tube marked LIGHT, add 10 drops of the light filtrate.

PROCEDURE: Continued

To the GLUCOSE test tube add 10 drops of the 10% glucose solution. To the DISTILLED WATER test tube add 10 drops of distilled water and to the test tube marked BEFORE add 10 drops of the dark filtrate. Place all the test tubes, except the BEFORE test tube, in a boiling hot water bath. Bring the contents of the four test tubes to a boil and continue to heat for 3 minutes. Observe the colors of the test tubes before and after heating. Use the BEFORE test tube as a comparison. To get a more accurate result use 2 cm pieces of Testape. Add 2 drops of each filtrate to the Testape. Note the color change, and staple these pieces of paper in your notebook.

RESULTS: Record your results in the following chart.

SAMPLE	BENEDICT'S SOLUTION		TESTAPE		OBSERVATIONS
	POS.	NEG.	POS.	NEG.	
BEFORE					
DARK					
LIGHT					
GLUCOSE					
DISTILLED WATER					

QUESTIONS:

1. Describe the results of each test tube in terms of chemical activity.
2. What was the purpose of the GLUCOSE, DISTILLED WATER and BEFORE test tubes in this experiment?
3. Was there a difference in color between the dark and light plant? If so, explain why?
4. What type of light can be used by plants to carry on photosynthesis?
5. Explain in full the function of the Benedict's solution.

INVESTIGATION 23

EXTRACTION OF PLANT PIGMENTS

PURPOSE: To show that carotenes, xanthophylls and chlorophylls occur in plants.

MATERIALS: Whatman #1 filter paper
rubber (or cork) stopper
mortar and pestle
sand (clean)
acetone
micropipette
solvent (9 parts petroleum ether and 1 part acetone)
curved pin or thumb tack
leaves of a geranium or other available leaves
50 ml beaker
scissors
test tube rack

PROCEDURE: Using a very small amount of clean sand, grind 3 leaves with 5 ml of acetone. Add an additional 5 ml of acetone if required. The resulting pigment extract should be as concentrated as possible. Decant the acetone-pigment extract into a 50 ml beaker. Discard the sand and plant material. Using a micropipette place a drop on the piece of filter paper that has been cut to fit the test tube. The spot should be applied 3-5 mm from the end of the filter paper. Reapply the spot 5 times allowing it to dry between applications. Make sure the filter paper does not touch the

PROCEDURE: Continued

test tube, otherwise a clear separation of the bands does not occur.

Using a curved pin, fasten the strip to the stopper. Place sufficient solvent in the test tube in order that the tip of the filter paper is in the solvent but the pigment spot is not below the surface of the solvent. Place the test tube in the test tube rack so that the filter paper does not touch the sides of the test tube and observe the movement of the solvent front. When the solvent is within 1 cm of the top, remove the piece of filter paper. Note the location of 4 pigments on the filter paper. Chlorophyll (a) will appear as a blue-green color, chlorophyll (b) a yellow green color, carotenes orange-red and xanthophylls will be light yellow. Measure the total distance the solvent travelled along the filter paper and the distance each pigment travelled. Calculate the (R_f) migration for each pigment, according to this formula.

$$R_f = \frac{\text{distance solute (pigment) travelled}}{\text{distance the solvent travelled}}$$

Record the R_f for each pigment in the following chart.

PIGMENT	R_f
Chlorophyll (a)	
Chlorophyll (b)	
Carotenes	
Xanthophylls	

QUESTIONS:

1. Which pigment travelled fastest? Explain why.
2. What is the specific role of chlorophyll in a plant?
3. Why does a geranium appear green?
4. Account for the change in color of leaves in the fall.
5. Explain the process of paper chromatography.
6. How would a different solvent affect the R_f values?

INVESTIGATION 24

LIGHT INTENSITY AND THE RATE OF PHOTOSYNTHESIS

INTRODUCTION:

The rate of photosynthesis is a function of light intensity, CO_2 concentration and temperature. This activity will demonstrate how light intensities affect photosynthesis.

PURPOSE: To investigate the effect of light intensity on the rate of photosynthesis of filamentous green algae.

MATERIALS: 10 x 100 mm test tubes
test tube rack
forceps
dissecting needle
aluminium foil
2 light sources
filamentous green algae and culture water

PROCEDURE: Fill 3 test tubes to 3/4 full with green algae and culture water. The algae should be densely packed but evenly distributed in the test tube. Cap the test tubes with aluminum foil and place them in separate test tube racks. Place one test tube in intense light (10 cm from the light source), the second test tube in low intensity light (40 cm from the light source) and the other in darkness. Check the temperature of the air directly in front of each test tube, if the temperatures vary place a beaker of water between the light sources and the test tubes. The water wil

PROCEDURE: Continued

absorb heat from the light source. Observe each test tube for the production of oxygen for the remainder of the class period and after 24 hours.

QUESTIONS:

1. What conclusions can be drawn between the intensity of light and a) the production of the gas? b) the rate of photosynthesis?
2. Explain how a difference in temperature among the three test tubes affects the results.
3. What other factors may influence the results of this investigation?
4. How could you determine what gas was given off?
5. What are the reactants and products of photosynthesis?

INVESTIGATION 25

CARBON DIOXIDE AND PHOTOSYNTHESIS

INTRODUCTION:

The atmosphere surrounding the earth is composed of many gases. The main constituents of the air are nitrogen, oxygen and carbon dioxide. In photosynthesis carbon dioxide is required and plants obtain the necessary CO_2 from the atmosphere.

PURPOSE: To demonstrate that CO_2 is necessary for the process of photosynthesis.

MATERIALS:
- 2 bell jars
- ethanol
- Petri dishes
- hot plate
- iodine solution
- KOH pellets
- beaker
- petroleum jelly or vacuum sealing wax
- 2 plants

PROCEDURE: Place two healthy plants in a dark cupboard for 2 days. Select one leaf from each plant and place them in a boiling alcohol bath until the chlorophyll has been removed. Place the stiff, white leaves in a petri dish and cover them with iodine solution. If the leaves do not turn blue-black continue with the experiment. If a blue-black

PROCEDURE: Continued

color appears, starch is present in the leaves and the plants must be kept in the dark cupboard for another day. Place one plant under a bell jar and seal the bottom edge with petroleum jelly. Place the other plant under the second bell jar along with a small beaker containing 50 ml of KOH pellets. Seal the bottom edge with petroleum jelly. Place both bell jars in a well lit location for three days and on third day remove a leaf from each plant and test for starch production.

QUESTIONS:

1. Why are the plants kept in a dark cupboard?
2. What function does the KOH serve?
3. What is the control in this exercise?
4. What is the experimental variable?
5. What can you conclude from your results? Suggest further experiments that would prove or disprove your conclusion.

INVESTIGATION 26

CHLOROPHYLL

INTRODUCTION:

Two of the few chemical units that may be activated by light and thus play a role in the conversion of light energy to chemical energy are the chlorophylls. The chlorophylls are located in chloroplasts and they are vital units in photosynthesis.

PURPOSE: To demonstrate that chlorophyll is necessary for photosynthesis to occur.

MATERIALS: silver leaf geranium or variegated coleus
ethanol
250 ml beaker
Petri dish
iodine solution
hot plate
forceps

PROCEDURE: Place the plant under direct light for 24 hours. Remove two or three leaves and immerse them in a boiling alcohol bath.The alcohol will kill the leaves and remove the pigments. When the leaf is stiff and white remove it from the alcohol bath and place it in a Petri dish. Pour iodine over the leaf and let it stand.

RESULTS: Sketch the outline of the leaf and shade in those areas that are high in chlorophyll and starch.

QUESTIONS:

1. What portion of the visible spectrum should have the least influence on the rate of photosynthesis? Explain your answer.
2. Purple hearts are plants that have a high concentration of anthocyanins which gives them a purple color. Explain how photosynthesis can occur in such a plant.
3. Alfonse, the "Mad Chemist", has developed "Anti Green". This chemical destroys chloroplasts and chlorophyll. Discuss the positive and negative effects this chemical will have if it has an active stage of ten years.

INVESTIGATION 27

DISSECTION OF A MAMMALIAN HEART

PURPOSE: To observe and study the structures and functions of the heart.

MATERIALS: Sheep or hog heart
scissors
scalpel
forceps
probe
dissecting tray

PROCEDURE: Obtain a heart with the longest possible attached arteries and veins. Place the heart on the dissecting tray with the apex towards you and pointing slightly to your right. The ventral surface should be slightly more convex than the dorsal surface but the heart may be distorted due to packing and preserving.

If the heart is covered with a membraneous sac, the pericardium, carefully remove it along with any fat at the top of the heart. Leave all associated blood vessels intact.

Note the positions and size of the two atria in comparison to the ventricles. The relative differences in the thickness of the walls of the atria and ventricles can be noted by feeling the walls of these chambers. Notice the thickness of the left ventricle in comparison to the right ventricle.

PROCEDURE: Continued

Note the following blood vessels:

Pulmonary artery. This is a thick-walled vessel leaving the right ventricle and is usually seen ventral to the aorta.

Aorta. This is also a thick-walled vessel leaving the left ventricle and bending to the right.

Vena cavae. These two blood vessels, the superior vena cava and the inferior vena cava, enter the right atrium anteriorly and posteriorly.

Pulmonary veins. These four veins are seen to enter the left atrium.

Coronary vessels. Two coronary arteries leave the aorta (to be seen during the dissection) and carry blood to the muscle tissue of the heart. Coronary vessels may be seen in the interventricular groove on the ventral surface of the heart.

Heart dissection

Make an incision from the top of the right atrium extending obliquely through the right ventricle. Do not cut completely through the tissue but leave the dorsal side intact so as to provide a hinge. It should now be possible to view the internal structure of the right atrium, tricuspid valve and the right ventricle. Observe the tricuspid valve and note the number of flaps comprising the valve, the attachment of the chordae tendineae and the papillary muscles to the flaps.

PROCEDURE: Continued

Make an incision into the pulmonary artery so as to expose the pulmonary semilunar valve. Make a similar incision from the left atrium to the left ventricle to view the internal structures of the chambers and the mitral valve. Note the same structures on this valve as you found on the tricuspid valve. Make a longitudinal incision into the aorta to expose the aortic semilunar valve and the openings to the two coronary arteries just above it. The wall dividing the right and left atria is called the interatrial septum. It may be possible to find a depression in this septum known as the fossa ovalis. This is the region of an opening that existed in the fetal heart prior to birth that allowed most of the blood to flow directly from the right to the left atria, by-passing the lungs.

QUESTIONS:

1. Using the dissected heart trace and describe the flow of blood through the heart. Include statements whether the blood is oxygenated or deoxygenated at specific locations.
2. Describe the structure and function of the valves and the associated chordae tendineæ and papillary muscles.
3. What is the reason for the difference in the thickness between the walls of the arteries and the veins as well as the left and right ventricles?

INVESTIGATION 28

THE HEART: SOUNDS AND PRESSURE

INTRODUCTION:

The "wringing out" action of the heart muscles results in the forced closing of two sets of heart valves. As a result, the heart sounds may be described as "lub-dub". The "lub" is due to the closing of the A-V valves, while the "dub" is caused by the closing of the semi-lunar valves which prevent back-flow from the pulmonary artery and the aorta when the ventricles are relaxed.

Pressure in the arterial reservoir will vary due to the action of the heart and the difference between the systolic and diastolic pressure. This pressure may be measured indirectly through the use of a sphygmomanometer and a stethoscope.

PURPOSE: To observe and record the influence various factors have on heart sounds, pulse rate and blood pressure.

MATERIALS: sphygmomanometer
stethoscope
cup of coffee
cigarette
metre stick
shallow pan

PROCEDURE: A) Heart sounds

Hold the bell of the stethoscope firmly against the chest of the subject (seated) at the fifth intercostal space and

PROCEDURE: Continued

about 8 cm to the left of the subject's midline. Listen to and describe the two heart sounds. Have the subject run on the spot for 2 minutes. Listen to the heart sounds again. Describe any change in the sounds.

B) Pulse rate

The pulse can be felt if an artery can be pressed against firm tissue. Normally the radial artery is most frequently used for feeling the pulse. When taking the subject's pulse use the index and one or two other fingers. After finding the pulse on the inside of the arm over the distal (wrist region) end of the radius count the pulse for one full minute. The pulse should be determined for the following six condition With the subject a) reclining, b) sitting, c) standing, d) running on the spot, e) immediately after exercise and f) with the subject's face in a shallow pan of water. Once this experiment has begun it must be continued until finished. Therefore, keep your fingers on the subject's pulse throughout the experiment. A partner will record the results and keep time. Check the affect of caffeine (coffee), smoking, hyperventilating and breath holding on pulse rate while the subject is seated.

C) Arterial blood pressure

With the subject seated and using a sphygmomanometer measure the blood pressure in the brachial artery in the inner elbow region. Attach the cuff, making sure it is slightly above the elbow at the lowest point. Raise the pressure to about 150

PROCEDURE: Continued

of mercury. At this point no sound should be heard with the stethoscope. Continue listening and slowly release the pressure until the first "tap" sounds are heard. Record the pressure at which this sound is heard. Continue to listen and release the pressure until the sounds become muffled or indistinct. Record this second pressure. The first reading represents the systolic pressure and the second reading represents the diastolic pressure.

Have the subject run on the spot for one minute and then record the subject's blood pressure after 1 minutes rest and after 3 minutes rest.

D) Venous blood pressure

Stand in front of the blackboard with a piece of chalk in your hand. Hold your arm at your side until the veins are distended. Raise your arm until it is at the level of the atria of the heart. The veins should still be extended. Mark the position of your hand on the blackboard. Have your partner slowly raise your arm until a height is reached where the veins are no longer distended. Mark this second position on the blackboard. Measure the distance between the two points in centimetres and record the measurement in the results section.

PROCEDURE: Continued

E) Valves of the veins

Examine your arm for venous valves. To locate the valves, low your hand to distend the veins. The sphygmomanometer may be used to help distend the veins. The valves will be indicated by slight swellings at points along the veins. With one finger press down on the distal (towards the hand) portion of a vein. With a second finger press the blood proximally (toward the body) beyond the next swelling. The vein should now be empty. Caution - do not force the blood in the reverse direction. Remove your second finger and observe the vein, now remove your first finger and observe the vein. Record your results under observations.

RESULTS: A) Heart sounds

Describe the heart sounds before and after exercise.

B) Pulse rate

RESULTS: Continued

ACTIVITY	TIME INTERVAL (MINUTES)	RATE (BEATS/MINUTES)
Reclining	1.	
	2.	
	3.	
Sitting	1.	
	2.	
	3.	
Standing	1.	
	2.	
	3.	
Running on the spot (followed by rest)	1.	
	2.(after rest of 1 min)	
	3.(after rest of 3 min)	
Brachycardia (face in shallow pan of water)		

INFLUENCE ON PULSE RATE OF:	RATE/UNIT TIME		
	ONE MINUTE BEFORE	ONE MINUTE AFTER	FIVE MINUTES AFTER
Coffee			
Smoking			
Breath holding			
Hyperventilation			

RESULTS: Continued

C) Arterial blood pressure

CONDITION	SYSTOLIC READING	DIASTOLIC READING
Seated		
One minute after Exercise		
Three minutes after exercise		

D) Venous blood pressure

Difference between two points = _____ cm

Use the following equation to calculate your venous pressure where:

$$V.P. = (d - 10) \times \left(\frac{1.055}{13.550}\right) \times 10 \text{ mm of Hg}$$

d = distance between two points,

1.055 = specific gravity of blood,

13.550 = specific gravity of mercury, and the venous pressure is partly dependent on the resistance of the veins which may be assumed to be equal to a 10 cm height of blood.

E) Valves of the veins

From your observations answer the following questions.

1) When the second finger is removed does the vein fill up completely? Explain why or why not.

2) Draw a diagram of a section of a vein and show the design of a valve.

QUESTIONS:

1. Using the data from part B (Pulse rate) calculate the class average for each second for the second time interval when the subject was seated.
2. From part C (Arterial blood pressure) calculate the class average for the systolic and diastolic pressures while the subject was seated.
3. Compare your readings for question 1 and 2 with the class average.
4. What conclusion can be reached about the effect the various factors discussed in this exercise had on your pulse rate and blood pressure?

INVESTIGATION 29

BLOOD ANTIGENS

INTRODUCTION:

In 1900, a young pathologist, Karl Landsteiner, discovered that human blood was not the same in all individuals. It was noticed that clumping would sometimes occur when blood from two individuals was mixed. Landsteiner classified human blood into four well defined groups.

Blood grouping is founded on the presence or absence of specific antigens (glycoproteins) called agglutinogens. These antigens are located on the protein layer of erythrocytes and they are designated by the symbols A and B. If a person has the A antigen he would have type A blood while a person with the B antigen would have type B blood. If the person has both antigens he will have type AB blood and if both antigens are lacking the person will have type O blood.

It is also known that group A people have an antibody (agglutinin) in their blood plasma. This antibody combines with the B antigen thus causing clumping. This anti-B antibody is called the β(beta) antibody while group B people have the α (alpha) antibody.

BLOOD TYPE	AGGLUTINOGENS on erythrocytes	AGGLUTININS in plasma	CAN DONATE BLOOD TO:	CAN RECEIVE BLOOD FROM:
A	A	β	A, AB	O, A
B	B	α	B, AB	O, B
AB	AB	None	AB	A, B, AB, O
O	None	α,β	A, B,AB,O	O

In 1940, Landsteiner, along with his cohort Weiner, discovered another antigen known as the "Rh factor". Individuals possessing the antigen are called Rh-positive (Rh^+), while those who lack the antigen are called Rh-negative (Rh^-).

Many other types of antigens have been found to occur in human red blood corpuscle membranes and they are important from the point of view of antigen-antibody reactions.

PURPOSE: To determine the A, B, O blood groups as well as the presence or absence of the Rh factor.

MATERIALS: 2 microscope slides
grease pencil
cotton
ethanol
Anti-A serum
Anti-B serum
Anti-Rh serum
toothpicks
incubator or glass plate and lamps
sterile blood lancets

PROCEDURE: Using a grease marking pencil, make a dividing line through the center of a thoroughly clean slide. Mark the left portion "Anti-A" and the right portion "Anti-B". The second clean slide is marked with an "Rh" in the upper right hand corner. Place the Rh slide in an incubator set at 37^oC. If an incubator is not available the slide should be placed on a glass plate warmed to 37^oC - 45^oC by electric lamps. Note - it is important that the slide by kept at approxi-

PROCEDURE: Continued

mately body temperature throughout the Rh antigen test. Thoroughly clean the tip of the middle finger with cotton dipped in ethyl alcohol. While applying pressure to the middle finger, use a sterile lancet to prick your finger. Caution - DO NOT use the lancet again, discard it immediately. Squeeze your finger to increase the blood flow and add a drop of blood to the slide marked "Rh". Follow the same procedure placing two drops on the slide marked "Anti-A" and "Anti-B". Clean the tip of your finger with cotton and alcohol and hold the cotton firmly over the puncture until the bleeding stops.

Place one drop of anti-Rh serum beside the drop of blood on the "Rh" slide. Also place one drop of anti-A serum beside the drop of blood on the "Anti-A, Anti-B" slide. Use 3 separate, clean toothpicks stir the anti-sera into the drops of blood. Let the slides stand for 2 minutes and observe the drops of blood for agglutination. Note - the agglutination with Rh serum does not produce large clumps of cells seen in the A, B, O blood tests. While rocking the slide gently back and forth look for a change in the mixture that produces a granular appearance rather than the original smooth suspension.

Agglutination should occur within 2 minutes. After this time clean or discard the slides.

RESULTS: Record your own blood type as well as the class results in the following chart.

BLOOD TYPE	INDIVIDUAL RESULT	CLASS RESULT	PERCENTAGE OF CLASS
A			
B			
AB			
O			
Rh^+			
Rh^-			

QUESTIONS:

1. To whom could you give blood for a transfusion?
2. From whom could you receive blood?
3. To whom should you not give blood? Explain fully.
4. Which blood type is most common in your class and which is the least common?
5. How closely does the data obtained in Question 4 compare with nationally accumulated data?
6. Which Rh type is most common in your class?
7. Why is it important to determine a person's blood type prior to a transfusion?
8. What fractions of the blood are considered when donors and recipients are matched?
9. How could you determine your blood type without the use of anti-sera?

INVESTIGATION 30

INHERITANCE OF BLOOD TYPES

INTRODUCTION:

It has been established that A, B, and O blood groups are dependent on three alleles which are located at a single gene locus. You may recall that everyone inherits allelic chromosome pairs with each allele bearing a gene for a particular blood protein. The genes are labelled L^A, L^B and L^O. The gene is labelled with an "L" for Karl Landsteiner who first discovered the A, B, O blood groups and the superscripts represent the type of antigens produced. In the case of L^O, no antigen is produced. If genes L^A and L^B are present on the alleles, then both genes are expressed. Therefore, because of nondominance (codominance), six different genotypic expressions can exist but only four phenotypic expressions can be observed.

GENOTYPE	PHENOTYPE	AGGLUTINOGENS	AGGLUTININS
L^OL^O	O	none	α and β
L^AL^O or L^AL^A	A	A	β
L^BL^O or L^BL^B	B	B	α
L^AL^B	AB	A and B	none

At the present time there is some confusion about the exact means of inheritance of the Rh antigens, it is now believed that a single pair of genes are responsible. Although there are six different Rh antigens

only one of these seems to be of major clinical importance. The Rh_o antigen is the one most often responsible for detrimental blood conditions produced by Rh incompatabilities.

The expression of the Rh factor is a dominant condition.

PURPOSE: To study the genetic basis of A, B, O blood groups.

MATERIALS: None

PROCEDURE: Answer the following questions

QUESTIONS:

1. What blood groups are not possible in the offspring of two AB parents?
2. What blood groups are not possible in the offspring of two O parents?
3. On the basis of your study of the inheritance of human blood types, is it possible to prove that a child belongs to certain parents? Explain.
4. What would the genotypes of the parents be if one parent is Group A and the other is Group B. All four blood groups are represented among their children.
5. In the following two cases of disputed paternity, determine the probable father of the child:
   a) The mother has type B blood, the child is Group O, one possible father is Group A and the other is AB.
   b) the mother is Group B, the child is AB, one possible father is Group A while the other possible father is Group B.

6) In the choice of donors for blood transfusions, a patient's brother or sister is often selected. Would a transfusion be more likely to succeed if both parents of the donor belonged to the AB blood group or if they both belonged to Group O? Explain your answer.

7) What is erythroblastosis fetalis?
Explain your answer in terms of antigen-antibody reactions.

INVESTIGATION 31

IDENTIFICATION OF HUMAN BLOOD CELL TYPES

INTRODUCTION:

Blood is a tissue composed of cells suspended in a liquid portion called plasma. Red blood cells (erythrocytes), white blood cells (leucocytes) and platelets are the three cell-like structures that are suspended in the plasma.

Red blood cells are concave on each side and they will appear to be ring-like due to the center being much thinner (1 μm) than the rim. These structures occur in the greatest number and their biconcave shape increases the rate at which gases are exchanged between the cell and the plasma.

Leucocytes are less numerous than red blood cells, there is approximately one leucocyte for every 700 erythrocytes. The white blood cells are somewhat larger than the erythrocytes and they are nucleated. Some leucocytes have granules in their cytoplasm, thus they are known as granular leucocytes.

Neutrophils, eosinophils and basophils are the three types of granular leucocytes. Each of these cells have features that can be used to distinguish them when the granules and nuclei are appropriately stained. In addition to the three granular leucocytes there are two nongranular leucocytes, the lymphocytes and monocytes. The blood platelets are the last type of cells found in the plasma. These cells are disc or irregularly shaped and they are non-nucleated. In size, platelets are much smaller than red blood cells.

Wright's stain which contains methylene blue and eosin is commonly used to stain blood cells. When the blood sample is spread in a thin layer over a microscope slide and stained the nuclear materials and a few granules stain blue, while other granules in the leucocytes stain red. The cytoplasm of the erythrocytes stain a pinkish color.

RED BLOOD CELLS

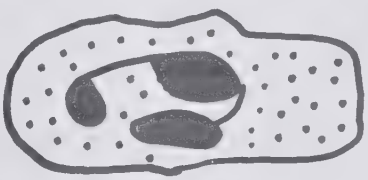
ERYTHROCYTES

- Most common
- Binconcave
- Non-nucleated
- Pinkish in color

GRANULAR LEUCOCYTES

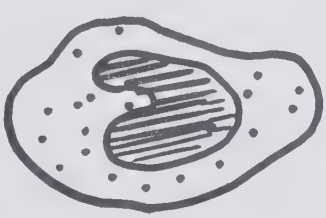
NEUTROPHILS

- Most common of the leucocytes
- Larger than red blood corpuscles
- Nucleus stains blue and consists of 2 or 3 lobes that are connected by fine strands.



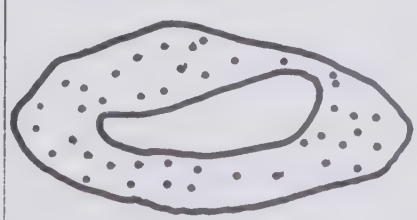
EOSINOPHIL

-About the same size as a neutrophil
-Nucleated
-Contains many bright red stained granules in the cytoplasm.



BASOPHILS

-Same size as a neutrophil
-Contain blue stained granules in cytoplasm
-Nucleus may not be visible



NON-GRANULAR LEUCOCYTES

LYMPHOCYTES

-Are slightly smaller than neutrophil
-Contain a large blue stained nucleus that almost fills the cell.

MONOCYTES

-Resemble lymphocytes except the large nucleus is bean-shaped in some cases.

PLATELETS

-Irregular or disc shaped
-Smaller than erythrocytes.

PURPOSE: To identify the various types of cell-like structures in a blood sample

MATERIALS: phosphate buffer of pH 6.8

sterile blood lancets

ethanol

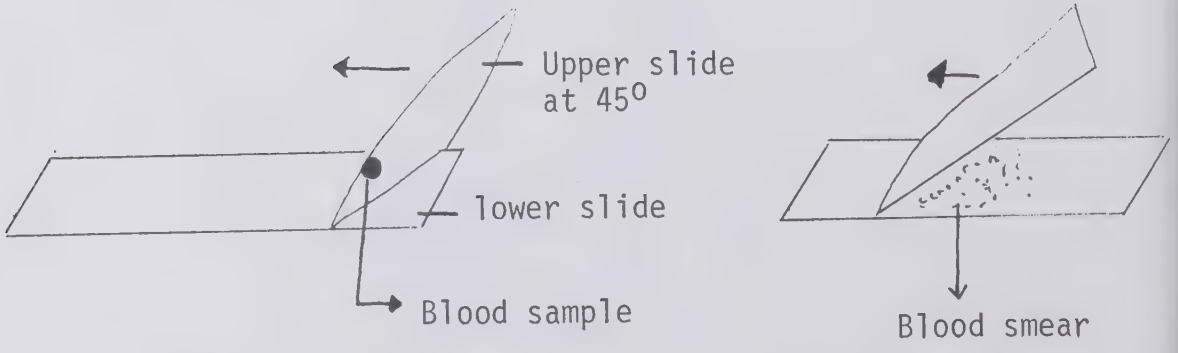
cotton

Wright's stain

microscope slides

microscope

PROCEDURE: Obtain two microscope slides and clean them thoroughly. After washing, handle the slides by their edges. Thoroughly sterilize the tip of one of your fingers with cotton dipped in ethanol. Using a sterile lancet prick the sterilized finger and place a drop of blood near the end of one slide. Hold the second slide at a 45^{o} angle to the slide with the drop of blood. Bring the edge of the second slide towards the drop of blood until it just comes in contact with the blood sample. Push the upper slide along the lower slide away from the drop of blood. This will spread the blood sample on the lower slide.



PROCEDURE: Continued

Allow the resulting blood smear to dry. The surface of the dried blood will appear dull. When the sample is sufficiently dry the blood smear is covered with freshly filtered Wright's stain. Allow the stain to remain on the slide for 2 minutes. At the end of 2 minutes add the phosphate buffer and mix the stain and the buffer by blowing on the slide. Stop adding the buffer when a gold metallic sheen appears. At this point there is a correct ratio of buffer to stain. Note - distilled water may be substituted in place of the phosphate buffer, however, the end result may not be as satisfactory. Let the slide remain undisturbed for 13 minutes. At the end of this time period gently tip the slide and wash the stain and buffer mixture off the slide with buffer. Next wipe the back of the slide with paper towelling. Stand the slide vertically and allow it to air dry. The blood smear can now be examined under the microscope but if oil immersion is used, the sample should be mounted under a cover slip.

RESULTS: Make sketches of red blood cells, leucocytes and platelets. Label granules and nuclei in the appropriate blood cells.

QUESTIONS:

1. What is the primary function of:

   a) red blood cells

   b) neutrophils

   c) lymphocytes

   d) monocytes and

   e) platelets

QUESTIONS: Continued

2. Do red blood cells appear to have a nucleus?
3. What does it mean when a person is anemic?
4. What is "pus" composed of?
5. Explain why the flow of blood usually stops quickly when you cut yourself.
6. Would you expect to get a positive DNA test in a mature red blood cell? Explain why or why not?

INVESTIGATION 32
IMMUNE REACTIONS

INTRODUCTION:

Agglutinins are specific proteins which are commonly found in the tissues of plants and animals. These proteins can combine with carbohydrates and glycoproteins (complex proteins containing carbohydrates in their structure). These agglutinins can combine at once with two or more carbohydrate groups thus clumping can occur.

PURPOSE: To demonstrate the immune response using tissue extracts from plants.

MATERIALS: fresh potato
peas
red kidney beans or lima beans
mortar and pestle
0.9 % NaCl
ethanol
cotton
sterile lancets
microscope slides
microscope
test tubes
test tube rack
eye droppers (clean)

PROCEDURE: Prepare a tissue extract of the potato by grinding a small piece with 1 ml of 0.9% NaCl in a mortar. Filter, decant

PROCEDURE: Continued

or centrifuge this mixture. A cloudy extract and a solid residue will result. Save the liquid extract and discard the solid residue. Label the test tube containing the potato extract and repeat the above procedure for the kidney beans or lima beans and the peas.

Next prepare a red blood cell suspension by pricking a clean sterilized finger with a sterile lancet. Wash the drop of blood into a clean test tube with 1 ml of 0.9% NaCl solution.

Obtain three clean test tubes and add 3 drops of the red blood cell suspension to each test tube. Using a clean eye dropper add 3 drops of the potato extract to test tube number 1, 3 drops of the red kidney bean extract to test tube number 2 and 3 drops of the pea extract to test tube number 3. Examine each of the three mixtures every 5 minutes by placing 1 drop of the mixture on a clean slide and observing it under the microscope.

RESULTS: Check each sample for clumping and note the time at which it occurs. Record your results in a suitable chart. Obtain the results of the class for the blood types (Exercise 32) that clump with plant extracts. The results should be recorded in the following manner.

RESULTS: Continued

SAMPLE	BLOOD TYPE			
	A	B	AB	O
0.9% NaCl				
Potato Extract				
Lima or Kidney Beans				
Peas				

QUESTIONS:

1. In this investigation what substances are acting as an antigens?
2. Offer an explanation as to how drugs suppress the body's immune response.
3. What would be a suitable control for this laboratory exercise?
4. Explain what the antigenic - determinant is and compare the antigen-antibody reaction to the enzyme - substrate complex.
5. Why is a newborn baby immune to several diseases for the first 6 weeks of its life?
6. What role do antigen-sensitive lymphocytes play in secondary immunity?
7. Devise an experiment that would determine which compound of the extracts are responsible for clumping.
8. What effect would the addition of papain (a proteolytic enzyme) have on the clumping action of blood and a plant extract?

INVESTIGATION 33

GAS EXCHANGE

NOTE - it is suggested that this exercise be set up as a number of stations to which students move in turn, in groups of two. One student acts as the recorder and the other as the subject. These roles should be reversed at each station so that data is collected for both students.

INTRODUCTION:

In man, the process breathing, is determined by his ability to change the pressure in the thoracic cavity. Muscles located between the ribs and a sheet of muscle known as the diaphragm are responsible for this change in pressure. When the external intercostal muscles contract this causes the rib cage to be pulled up and out. At the same time the diaphragm contracts and it is pulled downward. These two actions cause an increase in the size of the thoracic cavity and hence a partial vacuum is created and air is drawn into the lungs. When the external intercostal muscles and the diaphragm relax the size of the thoracic cavity decreases this causes a positive pressure in the thoracic cavity compared to the atmosphere and air is forced out of the lungs.

PURPOSE: To provide the student with the opportunity to study gas exchange in humans.

MATERIALS: 2 1000 ml graduated cylinders

battery jar

rubber tubing

"Y" connector

ethanol

plastic bags

stop watch or clock with second hand

microscope slides

ice water

stethoscope

500 ml beaker

100 ml beaker

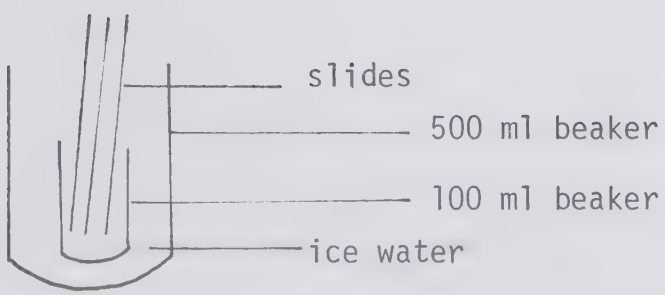
measuring tape

thermometer

cotton

PROCEDURE: A) Temperature Changes

Record the room temperature. Next hold the bulb of the thermometer between the lips of the subject, but do not touch the thermometer to the lips. Have the subject exhale and record the temperature of his breath. Hold a slide, previously cooled as shown, by the mouth and exhale onto it. Record your observations.



PROCEDURE: Continued

B) Mechanics

Using a tape measure record the circumference of the chest at the third rib, and the circumference of the abdomen at the level of the umbilicus when the subject inhales deeply as well as when he exhales deeply. Next place the bell of a stethoscope on the trachea of the subject and record your observations when the subject inhales deeply, exhales deeply and swallows.

C) CO_2 and rate of breathing

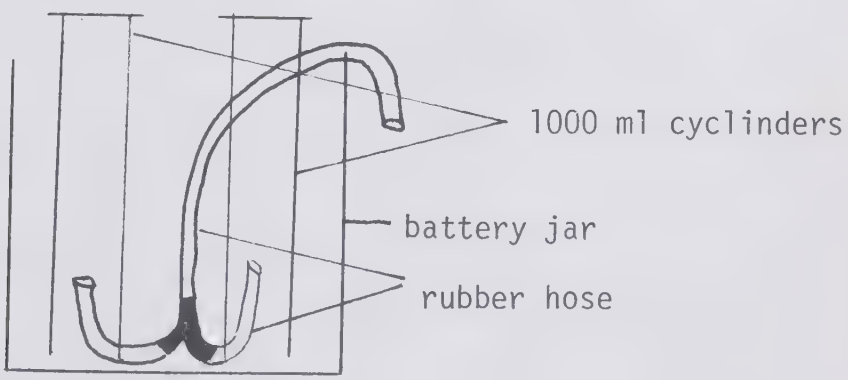
With the subject seated count the number of breaths in a 3 minute period. Calculate the average and record this in the results section. Next have the subject run on the spot for 2 minutes and then sit down. While he is seated count the number of breaths in a 3 minute period. Calculate the average number of breaths/minute and record this in the appropriate space in the results section. When the rate of breathing has returned to normal have the subject hyperventilate by breathing deeply at a rate which is faster than normal. Caution - watch the subject for dizziness. At the end of the last breath record the number of seconds before the next normal breath. Record the number of breaths for a 3 minute period and then calculate the average. Record these two results in the appropriate chart. When the subject's breathing has returned to normal have him place a plastic bag over his nose and mouth. Caution - watch the subject closely. Count the number of breaths in a 3

PROCEDURE: Continued

minute period and calculate the average. Record this value in the results section. Next calculate the average time between normal breaths by dividing 60 by the average number of breaths per minute. Record this value in the results section.

D) Lung capacity

Fill two 1000 ml graduated cylinders with water and invert them into a battery jar of water. Insert a piece of rubber tubing into each of the cylinders. The pieces of tubing are joined to a "Y" connector so that the subject may exhale into the two cylinders. The readings will involve the total displacement of water in each cylinder. The cotton and ethanol may be used to clean the "mouth piece".



After a normal inspiration breath into the spirometer. Record the volume of air that is trapped in the two graduated cylinders. This volume is known as the tidal volume. Refill the cylinders with water and then the

PROCEDURE: Continued

subject should exhale normally into the room and then exhale all the air that is left in your lungs into the spirometer. Record this volume as expiratory reserve. Next, refill the cylinders with water and inhale as deeply as possible. Exhale a normal breath into the spirometer and record the resulting volume as vital capacity. Subtract the value of the tidal volume from the vital capacity and record the difference as the inspiratory reserve.

RESULTS: Part A - Temperature Changes

SUBJECT	ROOM TEMP. (^{o}C)	BREATH TEMP. (^{o}C)	EFFECT ON SLIDE

Part B - Mechanics

SUBJECT	CHEST CIRCUMFERENCE (cm)	ABDOMEN CIRCUMFERENCE (cm)

RESULTS:

Part I - CO_2 Control On Breathing Rate

SUBJECT	BREATHS PER MINUTE			
	RESTING	AFTER EXERCISE	AFTER HYPER-VENTILATION	INTO BAGGIE

SUBJECT	TIME LAPSE FROM LAST BREATH IN HYPERVENTILATION TO 1ST NORMAL BREATH	AVERAGE TIME BETWEEN THE NORMAL BREATHS

Part II - Lung Capacity

SUBJECT	TIDAL VOLUME (ml)	EXPIRATORY RESERVE (ml)	VITAL CAPACITY (ml)	INSPIRATORY RESERVE (ml)

QUESTIONS:

1. Account for any difference in the temperature readings in Part A.
2. Explain the origin of the material that collects on the surface of the slide in Part A.
3. How is the thoracic cavity made longer? Broader?
4. How is the change in the size of the thoracic cavity related to inspiration and expiration?
5. From Part C, use CO_2 content as a guide, explain any differences between the number of breaths per minute seated and;
   a. the number per minute after exercise,
   b. the number per minute after hyperventilation
   c. the number per minute when the plastic bag was over the nose and mouth.
6. Compare the average time between normal breaths with the time lapse from the last breath of hyperventilation to the first normal breath. What does this indicate?
7. Using the data collected for Parts C and D, calculate the respiratory volume/minute for
   a. resting and
   b. after exercise

INVESTIGATION 34

THE AMOUNT OF CO_2 EXHALED IN A GIVEN TIME

PURPOSE: To calculate the amount of CO_2 exhaled in a given length of time.

MATERIALS: phenolphthalein (1 g dissolved in 40 ml of ethyl alcohol, dilute with 120 ml of distilled water)

glass tubing or straws

rubber stopper

2 Erlenmeyer flasks

burette

burette clamp

burette stand

0.1 M NaOH

PROCEDURE: Prepare two flasks by adding 3 to 5 drops of phenolphthalein to 100 ml of tap water in each flask. Make the solution in each flask slightly alkaline by adding a few drops of 0.1 M NaOH. Tightly stopper one flask to keep it from being exposed to the air.

Use a straw or a piece of glass tubing to gently exhale into the unstoppered flask. Record the time necessary to just have the pink color disappear. Run on the spot for 2 minutes and immediately exhale into the second flask. Record the time necessary for the pink color to just disappear.

Titrate each sample to determine the number of moles of CO_2 that were exhaled in one minute. Using a burette filled

PROCEDURE: Continued

with 0.1 M NaOH add the base drop by drop until the end point is reached and the pink color persists. Record the number of millilitres of NaOH that was required to reach the end point. Calculate the number of moles of CO_2 that were exhaled in 1 minute before and after exercising. Each millilitre of 0.1 M NaOH combines with 1×10^{-5} moles of CO_2.

RESULTS: Record your results in the following chart

FLASK	TIME (s)	NUMBER OF ml OF NaOH	MOLES OF CO_2 PRESENT
Number 1 Before Exercise			
Number 2 After Exercise			

QUESTIONS:

1. Write an equation explaining the change in pH of the water sample from the time it is colored to when it is colorless.
2. How does the time factor compare between flask 1 and 2? Explain any difference.
3. Why should the second flask be stoppered prior to use?
4. What affect should CO_2 have on your blood pH? Explain why this does not occur.
5. Why are resuscitators equipped with a mixture of 95% O_2 and 5% CO_2?

INVESTIGATION 35

CELLULAR RESPIRATION

INTRODUCTION:

Cellular respiration is the oxidation of organic compounds that occurs in cells. In general and much simplified terms the process can be summarized by a chemical reaction such as the oxidation of glucose:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$$

In this activity methylene blue will act as a hydrogen acceptor when glucose is oxidized and it will become colorless.

PURPOSE: To show that cellular respiration occurs in cells. Yeast cells will be used to demonstrate this process in the following laboratory activity.

MATERIALS: test tubes and a test tube rack
Bunsen burner or hot plate
ring stand and ring
250 ml beaker
10 ml graduated pipette
20% yeast suspension
distilled water
0.005% methylene blue solution
0.06 M glucose solution

PROCEDURE: Place 2 ml of glucose solution, 4 ml of distilled water and 2 ml of methylene blue solution into test tube number 1. Into test tube number 2 place 2 ml of glucose, 2 ml of distilled water, 2 ml of yeast and 2 ml of methylene blue. Into test tube number 3 place 2 ml of yeast suspension, 4 ml of glucose and 2 ml of methylene blue.

PROCEDURE: Continued

Into test tube number 4 place 2 ml of yeast suspension and heat in a hot water bath for 5 minutes. After heating, allow the yeast cells to cool and add 2 ml of distilled water, 2 ml of glucose and 2 ml of methylene blue.

Time each test tube from the time the solutions were mixed until the blue color disappears.

Record your results in a chart similar to the one below.

TEST TUBE #	TIME FOR DECOLORIZATION
#1	
#2	
#3	
#4	

QUESTIONS:

1. How do you account for the differences in time for decolorization to occur?
2. What was the purpose of leaving the yeast suspension out of test tube number 1.
3. Account for the result noticed in test tube #4.
4. Suggest a method to extract the dehydrogenase from yeast cells.
5. What are the two major oxidizing agents in cells?
6. What is the opposite reaction of oxidation, explain why this opposite reaction is absolutely essential for cellular respiration to go on.

INVESTIGATION 36

OXYGEN CONSUMPTION DURING AEROBIC CELLULAR RESPIRATION

INTRODUCTION:

Cellular respiration is a process by which cells break down organic molecules and store the chemical energy in the form of ATP. This represents a chemically destructive process. In most cells, this process occurs in the stalked particles of the mitochondria. Most organisms require energy for carrying out the process of cellular respiration. When respiration requires oxygen it is known as aerobic cellular respiration. If an organism can carry on respiration without the use of ozygen it is said to be undergoing anaerobic cellular respiration. This latter process is often referred to as alcoholic fermentation due to the production of various alcohols as end products.

PURPOSE: To measure the rate of oxygen consumption in germinating seeds.

MATERIALS:

- germinating peas
- cotton
- test tubes
- KOH pellets
- Congo red dye
- 250 ml beaker
- ring stand and ring
- Bunsen burner or hot plate
- rubber tubing
- glass tubing
- pinch clamps
- capillary tubing (pre-bent)
- two-holed rubber stopper
- millimetre ruler
- test tube clamps

PROCEDURE: Obtain sufficient germinating seeds to fill a test tube. Divide the seeds into two groups. Gently boil one group of seeds for 8 minutes. Once the seeds have boiled set them

PROCEDURE: Continued

aside to cool.

While one half of the seeds are boiling a volumeter is set up in the following way. Half fill a test tube with the remaining seeds and place 1 cm of loose cotton on top of the seeds. Add 1 cm of KOH pellets above the cotton. (Caution - KOH is extremely corrosive - DO NOT touch the KOH to your skin or clothing).

Next insert a piece of glass tubing, 5 cm in length, into a two-holed rubber stopper. Attach a 5 cm piece of rubber tubing to the glass tubing. Place a pinch clamp on the piece of rubber tubing. (Caution - your instructor will demonstate the correct procedure for inserting glass tubing into rubber stoppers).

Insert the short end of a piece of right angled capillary tubing into the second hole of the rubber stopper. Attach a piece of cardboard (or a ruler), with millimetre markings, to the long arm of the capillary tubing. Once the stopper has been set up insert it in the test tube.

A second volumeter is prepared in the same manner except the boiled seeds are used. Clamp the volumeters to a ring stand so that the capillary tubing, with the millimetre guide, is level.

Open both pinch clamps and inject 1 cm of dye into the open ends of the capillary tubing. A syringe may be used for this purpose. Adjust the pinch clamps and let the volumeters stand for 2 minutes until equilibrium has been established.

PROCEDURE: Continued

Note the starting position of the outer end of the dye and record its movement at 30 second intervals for 10 minutes.

RESULTS: Record the results in a suitable table and plot the time (minutes) versus the distance travelled (mm) on a graph. The time is plotted on the X axis and the distance on the Y axis.

QUESTIONS:

1. What relationship exists between oxygen consumption and the boiled and non-boiled seeds?
2. Why should the seeds used in the control apparatus be boiled?
3. Why is it necessary to remove the CO_2 from the volumeters?
4. Why did the dye move in the direction it did?
5. Under what conditions might the dye move away from the test tube?

INVESTIGATION 37

KIDNEY STRUCTURE

INTRODUCTION:

The kidneys are bean shaped organs about the size of a clenched fist. They are attached to the body cavity by mesentery. Each kidney is embedded in fat which helps cushion them from shock as well as hold them in place.

The kidney is structurally subdivided into three areas: the renal cortex which makes up the outer portion of the kidney, the renal medulla which represents the inner two-thirds of the kidney and the renal pelvis which is a sac like cavity. The renal pelvis empties its contents into the ureter which leads to the urinary bladder.

Each kidney is composed of approximately 1,000,000 nephrons. The nephrons are the functional unit of the kidney. Each nephron is a filtration unit which controls the composition of the urine. The blood is brought by paired renal arteries to the kidneys where it is filtered and then it is carried away from the kidneys by paired renal veins. Structurally each nephron consists of a small cup shaped structure called Bowman's capsule. A small, winding proximal tubule leads from each capsule and becomes the loop of Henle which then leads to the distal tubule. The distal tubule fuses with the collecting tubule whose contents empty into the renal pelvis.

PURPOSE: To study the structure of a mammalian kidney.

MATERIALS: preserved sheep or hog kidney
kidney model
prepared slides of kidney in cross section
prepared slides of kidney in longitudinal section
microscope
dissecting trays
scalpel
probe

PROCEDURE: Obtain a preserved kidney and note the shape, color, fat deposits, hilus, blood vessels and ureter. Sketch and label the above noted structures. Carefully bisect the kidney longitudinally beginning at the hilus. Note the outer renal cortex, the inner renal medulla and the renal pelvis. The medullary portion of the kidney is composed of wedge-shaped structures called the renal pyramids. Sketch and describe the previous four structures.
Using a prepared slide of the kidney locate the renal pelvis. Describe the structures observed and make a sketch of a nephron and label Bowman's capsule, proximal tubule, loop of Henle, distal tubule and the collecting tubule.

QUESTIONS:

1. What structures are located in the renal pyramids?
2. In what portion of the kidney are the renal pyramids located?
3. Where does the ureter originate?
4. What structures are found in the renal cortex?
5. What structures are found in the renal medulla?
6. What major blood vessels bring blood to and carry blood away

from the kidney.

7. List the two major functions of the kidneys.

INVESTIGATION 38

COMPOSITION OF URINE

INTRODUCTION:

It has been noted that certain compounds are constantly found in urine while other substances rarely occur. For example nitrogenous wastes such as urea are always found in human urine, while nutritionally important substances glucose, proteins, and amino acids are very rarely found in the urine.

If the nephrons are functioning properly the composition of a urine sample will fall within a certain range. Due to this feature, physical examinations often include the testing of a urine sample.

PURPOSE: To determine some components of urine.

MATERIALS: fresh urine - this experiment requires that urine be collected immediately on awakening in the morning prior to ingestion of any food or liquid.

Collect the urine in a container and stopper tightly.

test tube rack	Bunsen burner or hot plate
test tubes	dilute NH_4OH
centrifuge tubes	pH paper or pH indicator
centrifuge	ring stand and ring
pipette	concentrated HNO_3
microscope	0.5 M $AgNO_3$ solution
microscope slides	dilute acetic acid
cover slips	0.25 M $MgSO_4$ solution
0.25 M $BaCl_2$ solution	Benedict's solution, Testape or Clinitest tablets

PROCEDURE: A) Solids

Pour a 15 ml sample of urine into a centrifuge tube. Note the color of the urine sample in comparison to other samples in the class. Explain any differences in color. Centrifuge the sample for 2 minutes. After centrifuging, pour the upper clear supernatant into a waste container. With a fine pipette, remove some of the sediment from the centrifuge tube and place 2 drops of the sediment on a clean microscope slide. Cover the sediment with a cover slip and examine it under a microscope.

A number of substances may be located, for example uric acid crystals, epithelial cells, blood cells and casts. Sketch and record any solid particles observed. Note the texture, size, shape and the color of the particles observed.

B) pH

Use a 10 ml sample of fresh urine and test the pH with pH paper. Record the pH level in the results section.

C) Chemical composition

Glucose

Place 5 ml of Benedict's solution in a clean test tube and add 10 drops of urine. Place the test tube in a hot water bath for 3 minutes and then remove and allow to cool. Note and record any color change. Testape or Clinetist tablets can be substituted for the Benedict's solution. A sample of urine from a diabetic could also be used for a comparison. The identity of the donor should be protected. Notice the odor of the diabetic sample. Note - the diabetic sample

PROCEDURE: Continued

C)

should be collected just prior to the administration of insulin.

Protein

Carefully add 5 ml of concentrated HNO_3 to a test tube. Caution - HNO_3 is very corrosive. Slant the test tube and very carefully add 3 ml of urine so that it floats on the surface of the acid. Observe the sample, paying particular attention to the interface of the urine and acid. Record your observations.

Chlorides

Place 5 ml of urine in a test tube and add a few drops of dilute HNO_3 to make the sample slightly acidic. Test the sample with pH paper to see if more acid is required. To this acidified sample add 7 drops of 0.5 M $AgNO_3$ solution. Observe and record your results.

Sulphates

To a 10 ml sample of fresh urine add dilute acetic acid until the solution is slightly acidic. Test the sample with pH paper as you did in the previous test. To the acidified sample add 7 drops of 0.25 M $BaCl_2$ solution and record any change in the sample.

Phosphates

Add 15 ml of fresh urine to a test tube and add sufficient dilute NH_4OH to make the solution slightly alkaline. Add

PROCEDURE: Continued

C)

20 drops of 0.25 M $MgSO_4$ solution. Set the test tube aside and observe after 24 hours. Examine samples of the solution by placing a few drops on a microscope slide. Sketch any crystals that you observe.

RESULTS: Record your results in the following chart.

T E S T	OBSERVATIONS
Solids	
pH	
Glucose	
Protein	
Chlorides	
Sulphates	
Phosphates	

QUESTIONS:

1. What is the major component of urine by volume?
2. What structure controls the chemical composition of urine?
3. What is a kidney stone? How does one form?
4. What happens to the functional units of the kidney of a person who has diabetes mellitus?
5. What is glomerulonephritis (Bright's disease)?

QUESTIONS: Continued

6. What is pyelitis?
7. What affect would a change in the blood pressure have on the production of urine?
8. Is the presence of glucose in the urine an indication of a pathological problem?
9. What would the protein found in a urine sample be? Where does it come from?

ADDITIONAL INFORMATION

Benedict's test

Light green	trace - 0.25 % glucose
yellow-green	0.25 % - 0.5 % glucose
yellow	0.5 % - 1.0 % glucose
orange	1.0 % - 2.0 % glucose
brick-red	over 2.0 % glucose

Protein test for albumen

A thin white layer of coagulated protein may appear at the interface of the nitric acid and the urine. Presence of protein is quite common in adolescence, it is of no significance.

Phosphate test

Crystals of ammonium magnesium phosphate will form if the phosphate ion is present.

INVESTIGATION 39

GIBBERELLIN AND ITS EFFECT ON PLANT SEEDLINGS

INTRODUCTION:

During the 1930's Japanese scientists discovered a fungus growing or rice seedlings. This parasite caused the elongation of the seedling and hence the name "foolish seedling" developed. Gibberellins have a marked effect when they are applied to many "dwarf" plants. This plant hormone causes the internodes to lengthen thus increasing the length of the plant. Gibberellins affect sprouting of buds, seed germination and the development of flowers in certain plants. In this exercise two types of pea seedlings are used to demonstrate the effect of gibberellins.

PURPOSE: To determine the effects of gibberellin on two types of pea seedlings.

MATERIALS: Little Marvel and Alaska Peas
vermiculite
germination trays
flower pots
gibberellic acid solution 100 mg/ℓ
(dissolve 0.1 g in 3 ml of ethyl alcohol and then add 1 ℓ of distilled water)
spray atomizers

PROCEDURE: Germinate 20 Little Marvel and 20 Alaska peas in separate germination trays. Place the seeds between moistened paper towels and cover until the seeds have germinated. Prepare a mixture of potting soil or vermiculite and add it to

PROCEDURE: Continued

several flower pots. Transfer the germinated seeds to the flower pots. Do not mix the types of seeds in the flower pots. Cover the seeds with 1 cm of soil or vermiculite and keep them moist.

Divide each variety of seedlings into two groups, labelling one "experimental" and the other "control". Place the pots so that they receive the maximum amount of light and they have similar temperatures. When the seedlings are several centimetres high, measure and record the height of each plant in each of the four groups (Little Marvel experimental, Little Marvel control, Alaska experimental and Alaska control,) in millimetres. Measure the height from the surface of the soil to the end of the apical bud.

Prepare two hand spray atomizers, one with gibberellic acid and the other with distilled water. Following each day's measurement, spray the experimental plants with the gibberellic acid and the control plants with distilled water. The groups of plants should be sprayed so that the possibility of drifting is avoided. Spray the plants uniformly so that the leaves, stems and apical buds are moist. Continue to record the height of each plant and spray daily for the next five days.

RESULTS: Record the change in height in a suitable chart.

QUESTIONS:

1. Graph the data from the four groups of plants. If the plants kept growing at their present rate what would the average height be for each group at the end of 10 days?

2. What conclusions can be drawn regarding the affects of gibberellin on each variety of peas?

3. What relationship may exist between the genetic trait of dwarfism in Little Marvel peas and gibberellin.

INVESTIGATION 40

NERVOUS COORDINATION: STRUCTURE

INTRODUCTION:

The brain is the master organ of our body and the nervous system is the network of nerves through which the brain receives and sends out electrical impulses. The brain is well protected by a number of membranes, fluid, bone and skin. There are three membranes that surround the brain, the pia mater is the membrane next to the brain and it is richly supplied with blood vessels. The middle membrane is the arachnoid mater while the outermost membrane is the dura mater. Located between the arachnoid mater and the pia mater is the cerebrospinal fluid which provides a cushioning effect for the brain. The three membranes are known collectively as the meninges. Further protection for the brain is provided by the bony cranial cavity.

The spinal cord is the second structure of our central nervous system (CNS) and it runs from the base of the brain down through the vertebrae. Nerves, composed of many neurons, attach directly to the brain or to the spinal cord. Cranial nerves are those that travel directly to the brain while spinal nerves innervate the spinal cord. Nerves can be further broken down according to the type of message they carry. Sensory nerves carry messages to the CNS while motor nerves carry messages out to the organs of the body. Occasionally some nerves serve a dual purpose, they are known as mixed nerves.

PURPOSE: To study the external and internal structure of a mammalian brain.

MATERIALS: sheep brain

dissecting tray

scalpel

scissors

forceps

probe

model of a human brain

PROCEDURE: A) External structure

Using the following set of instructions obtain a whole sheep brain and identify the cerebrum, cerebellum, medulla oblongata, mid brain and pons.

The largest portion of the brain is the cerebrum and it is composed of two cerebral hemispheres. Describe the appearance of the left and right hemispheres. The convolutions noticed are comprised of gyri, the crests, and fissures or sulci, the dips. Note any membranes surrounding the two hemispheres. Grasp the membranes with a pair of forceps and cut through the membrane so that a 2 cm square of tissue is removed from the surface of the brain. Compare the three membranes and describe them.

Observe the dorsal surface of the brain and note the longitudinal fissure which separates the two cerebral hemispheres. Force the two hemispheres apart and note the corpus callosum. This structure is the largest commissure of the brain. What is the function of this structure?

The structure that lies posteriorly to the cerebral hemi-

PROCEDURE: Continued

spheres is called the cerebellum. Describe this structure. Comparing the cerebellum of the sheep to the human model notice the differences. In man the cerebellum has lateral lobes with a medial structure known as the vermis.

Locate the medulla oblongata which is the portion of the brain that is continuous with the spinal cord.

Gently separate the occipital lobes of the cerebrum and pull back on the cerebellum. The anterior protrusion represents the pineal body of the forebrain and the posterior swelling represents the corpora quadrigemina of the mid-brain.

Observe the ventral surface of the brain. Locate the pons. It is found ventrally from the division between the posterior portion of the cerebral hemispheres and the anterior portion of the cerebellum. Note the stumps of a number of cranial nerves which attach to the pons and the medulla.

Observe the anterior portion of the ventral surface of the brain and locate a pair of bulbous structures projecting forwards. These are the olfactory bulbs. These structures are responsible for the sense of smell. What type of nerve conducts impulses to the olfactory bulbs? What would the name of this nerve be?

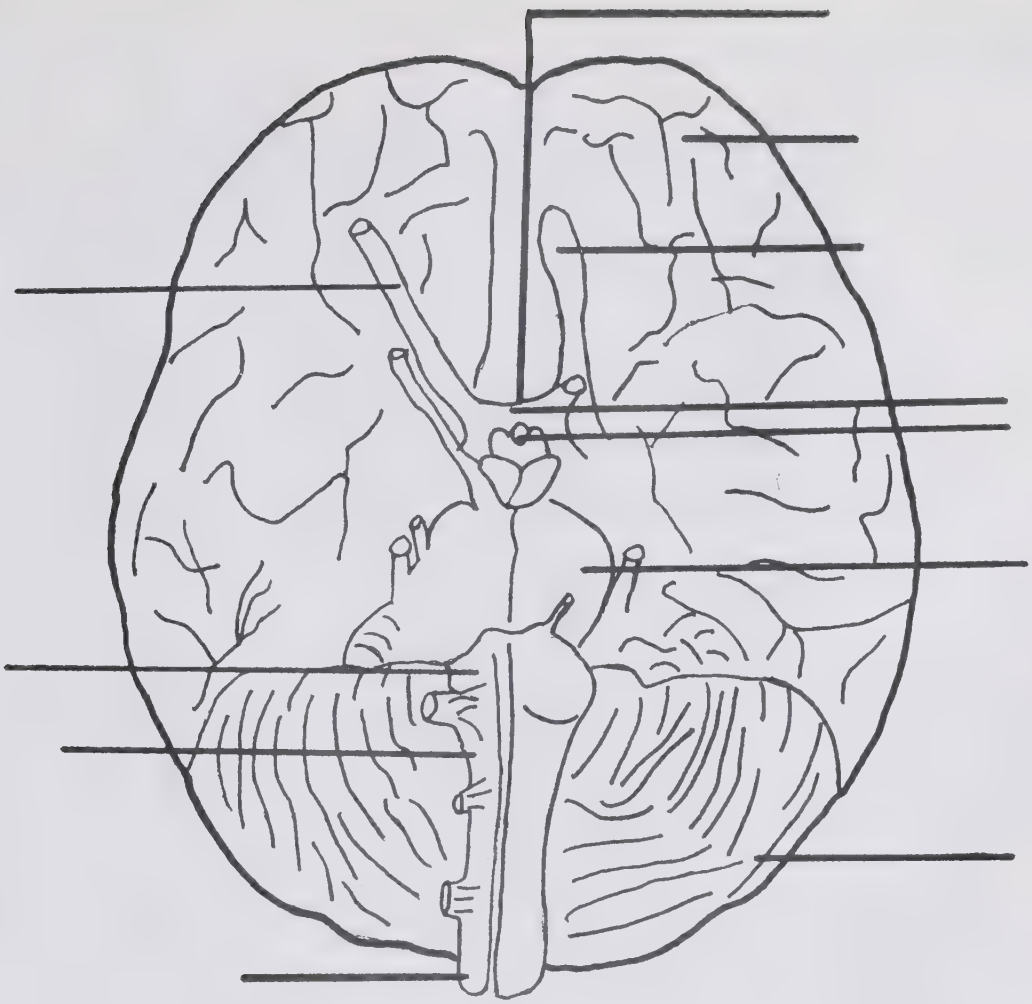
Move posteriorly along the longitudinal fissure until a white X-shaped structure is encountered. This is called the optic chiasma and they are the remnants of nerves coming from each eye. The occipital lobes are the portion of the

PROCEDURE: Continued

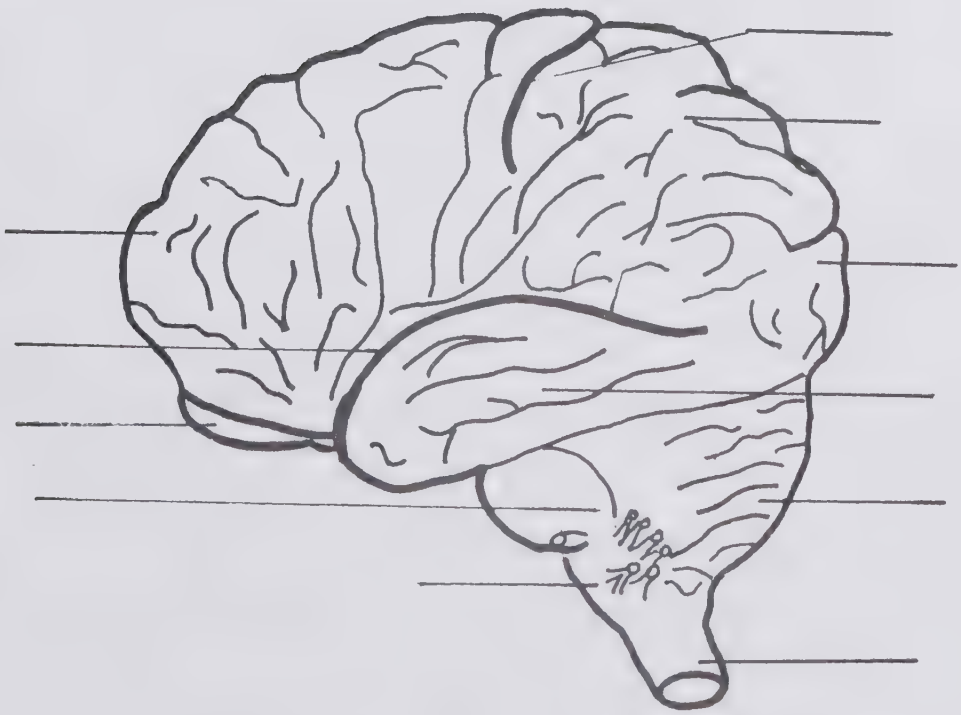
brain that receives the impulses from the optic nerves. What function would the occipital lobes have with respect to these impulses?

Lying posterior to the optic chiasma is the pituitary gland. Sometimes a piece of the sphenoid bone is left attached to the brain near this gland. If the gland is attached to the brain there is a short stalk called the infundibulum which separates the gland proper, from the brain.

Study a model or chart of a human brain. Locate the fissure of Rolondo which separates the frontal and parietal lobes of the cerebrum. Also locate the lateral fissure of Sylvius which separates the temporal lobe from the frontal and parietal lobes. Locate the occipital, temporal, frontal, olfactory, and parietal lobes. Label these structures on the accompanying diagram.



VENTRAL VIEW SHEEP BRAIN



LEFT LATERAL VIEW HUMAN BRAIN

PROCEDURE: Continued

B) Sagittal view of the sheep's brain.

Observe one half of a sheep brain that has been dissected in the sagittal plane by your instructor and locate the following parts.

Note the corpus callosum, the large white commissure which is located between the cerebral hemispheres. Insert a probe into the hollow area of the corpus callosum. Where does it lead? This is one of the lateral ventricles. Notice the color of the cerebral cortex in comparison to the color of the central portion (corpus callosum). Why does the color differ and how does it compare to the spinal cord? The thalamus is a nerve center located just beneath the round commissure, which is the region just posterior and ventral to the corpus callosum. The round commissure connects the right and left thalmic areas of the brain. Locate the pituitary gland (if present) on its stalked infundibulum, just ventral to the round commissure. The stalk of the pituitary is attached to the hypothalamus. Note the optic chiasma lies anterior to the infundibulum. The third ventricle appears as a groove running posteriorly from the middle of the round commissure. Run a probe along the groove in a posterior direction until a constriction occurs. This constriction is referred to as the aqueduct of Sylvius. This duct connects the third ventricle to the fourth ventricle. Follow the aqueduct of Sylvius until it

PROCEDURE: Continued

widens out and becomes the fourth ventricle. What constitutes the first and second ventricles? The large, convoluted structure just above the fourth ventricle is the cerebellum. Describe the sagittal view of this structure. Follow the fourth ventricle posteriorly and notice it becomes narrow and it is called the cerebrospinal canal of the spinal cord. Are all the ventricles continuous? What do the ventricles and cerebrospinal canal contain?

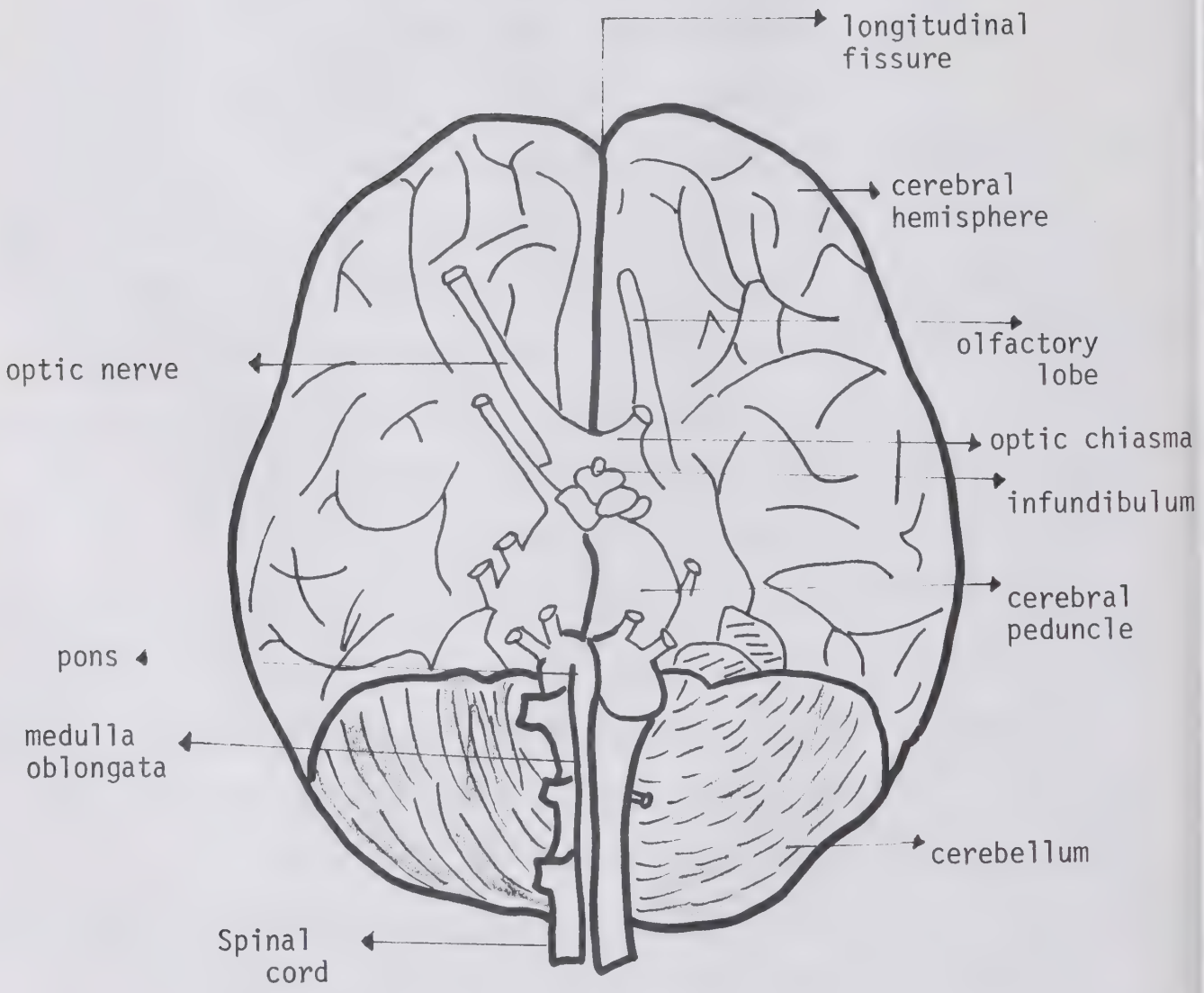
The area ventral to the fourth ventricle is the medulla oblongata and the area immediately anterior to the medulla is known as the pons.

Immediately above the aqueduct of Sylvius are anterior and posterior bodies or dorsal outgrowths of the midbrain. The cerebral peduncles, also representing the ventral portion of the midbrain, are represented by the tissue just anterior to the pons.

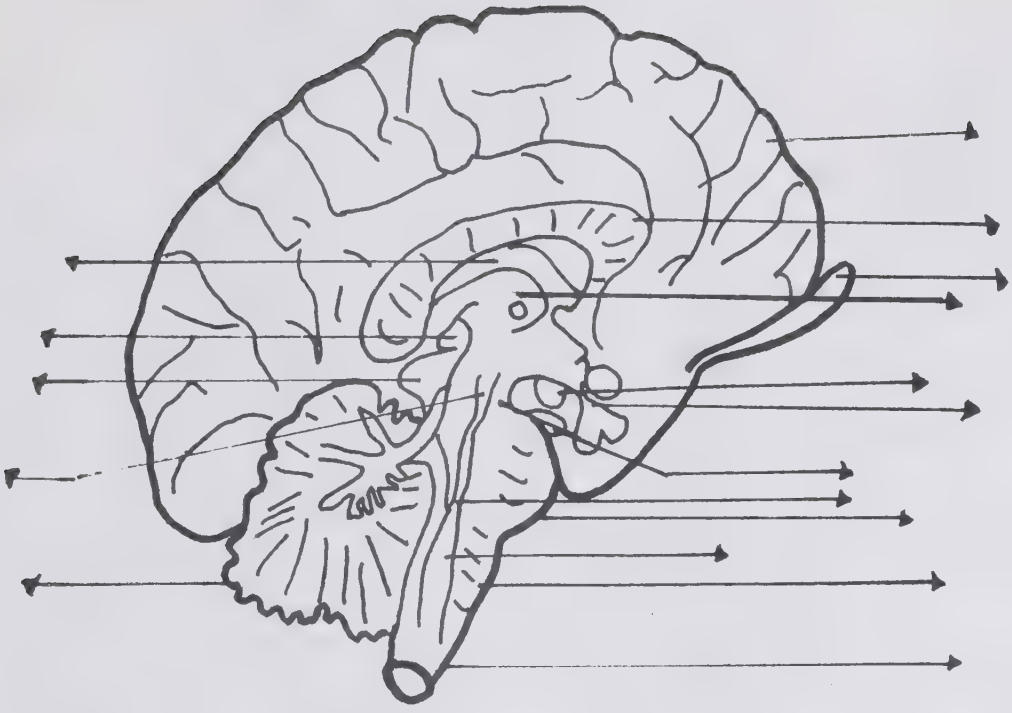
Label the sagittal view of the sheep brain on the accompanying diagram.

QUESTIONS:

1. What might be expected to be the primary signs of damage to the temporal lobe, cerebellum, and the medulla oblongata?
2. Name the three membranes surrounding the brain and the function of each membrane.
3. How is the surface area of the cerebrum increased without an increase in the total volume of the brain?



VENTRAL VIEW OF THE SHEEP BRAIN



Sagittal View Sheep Brain

INVESTIGATION 41

THE EFFECTORS - MUSCLES

INTRODUCTION:

Muscles are the organs of the muscular system. Muscles are categorized in three groups, namely striated, heart and smooth muscles. Muscles have developed the ability to convert chemical energy to mechanical energy. As a result man has the ability to move himself as well as to move other objects from place to place. Muscle tissue is quite efficient as it converts 40% of the potential energy the body receives into kinetic energy.

PURPOSE: To provide the student with the opportunity to observe muscle tissue and the action of muscles under varying conditions.

MATERIALS:

microscope	heart tissue	probes
lean meat		beakers
intestine		microscope slides
0.1% chromic acid solution		cover slips
20% HNO_3 solution		picrocarmine dye
ice		0.9% NaCl solution
sphygmomanometer		500 g mass

PROCEDURE: A) Muscle tissue

Use commercially prepared slides or place a small piece of lean striated and heart muscle in 0.1% chromic acid for 20-24 hours and a small piece of stomach or intestine in 20% HNO_3 for 48-72 hours. Once the muscle tissue has soaked for the appropriate length of time, tease the fibres

PROCEDURE: Continued

A) Muscle tissue

apart in 0.9% NaCl solution. Once the fibres are separated stain them with picrocarmine dye. Observe the three types of muscle tissue under high power. Sketch and label a diagram for each muscle type. Note the shape of each cell and the location of the cell nucleus.

B) Isotonic and Isometric contractions

Sit close to a lab bench or table and place your arm in a resting position, palm up on the table top. On the palm and fingers place an object with a mass which is too great to lift. With your elbow on the table attempt to lift the object. This is an isotonic contraction. Describe the changes in the upper arm muscles from relaxation to contraction.

Replace the object used in the previous section with a 500 g mass. Place the mass on the palm of your hand and lift it. Describe the changes in the upper arm muscles when an isometric contraction occurs.

C) Temperature

On a clean sheet of paper write your complete home address four times. Note the time in seconds it takes you to do this. Next hold ice cubes in your writing hand for the same length of time it took you to write your address four times. Immediately write your address four times. Rub your hands together briskly to return the temperature to normal and write your address four more times. Compare

PROCEDURE: Continued

C) Temperature

the results of the three sets of your address.

D) Fatigue

Over a 30 second time span count the number of times you open and close your hand rapidly. Repeat this 10 times and record your results. After a 3 minute rest repeat this activity but this time apply a sphygmomanometer to the upper arm. Apply sufficient pressure to the cuff so that the radial pulse can not be felt. Caution - you may have to reduce pressure on the cuff from time to time. Record the results in a suitable chart and graph the results for both trials on the same graph. The horizontal axis should be the number of trials and the vertical axis is the number of closures per trial.

E) Skeletal muscle

Expose the biceps of one arm. Relax the muscle, attach a strip of paper so that it is around the belly of the muscle. Flex the arm strongly upward at the elbow. Follow this by flexing the arm downward at the elbow. Notice the effect on the paper during movement of the arm in both directions.

QUESTIONS:

1. Describe skeletal, smooth and heart muscle.
2. Do isotonic and isometric contractions require energy?
3. Which type of contraction produces work?
4. Give a definition of isotonic and isometric contractions?

QUESTIONS: Continued

5. What effect does temperature have on musclar contractions?
6. What influence does a fresh supply of blood have on muscle fatigue?
7. Explain how a muscle changes length when it contracts.
8. What is meant by antagonistic muscles? Give an example.

INVESTIGATION 42

THE EAR AND BALANCE ORGANS

INTRODUCTION:

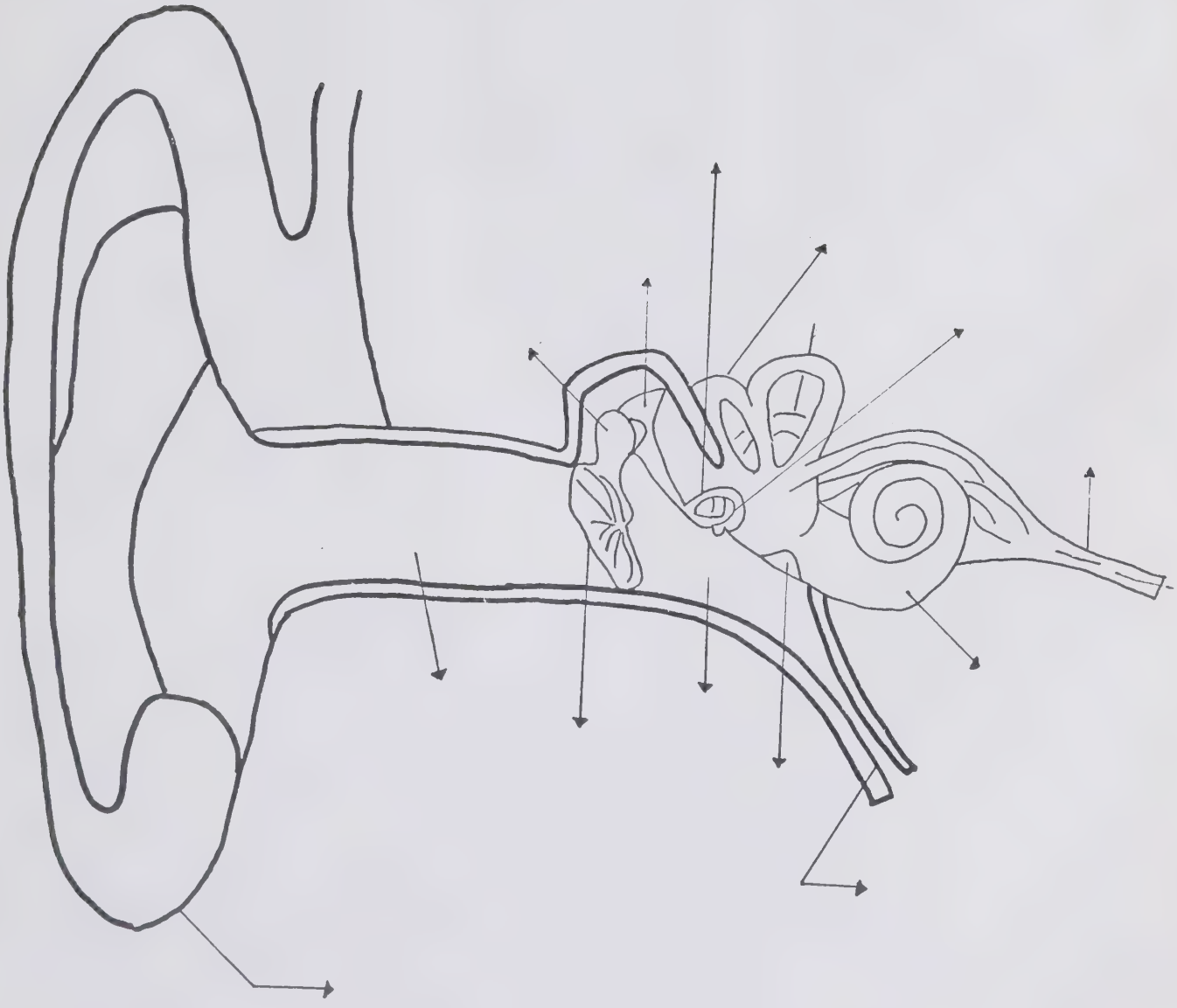
The auditory mechanism responds to sound waves. The ear is divided into three parts, the outer, middle and inner ears. These parts contain the hearing apparatus and the inner ear also contains the structures concerned with balance and equilibrium.

PURPOSE: To study the structure and function of the ear.

MATERIALS: model or chart of the ear
swivel chair
waste basket
clock
watch
metre stick
tuning fork

PROCEDURE: A) Structure of the ear

From a model or chart of the ear identify the following structures, pinna (auricle), external meatus, tympanic membrane (eardrum), malleus, incus, stapes, middle ear cavity, round window, oval window, cochlea, semicircular canals, auditory nerve, and the Eustachian tube. Label the following diagram.



PROCEDURE: Continued

The subsequent activities should be done in a quiet area: Work in pairs.

B) Auditory activities

The subject covers his left ear with his left hand and stands approximately 8 m from his partner. The partner stands on the side of the uncovered ear with a clock. Hold the clock at arm's length and keep moving closer to the subject until the ticking of the clock can be distinguished. Record this distance in metres. Repeat the procedure for the other ear and record the distance. The roles should be reversed so that auditory acuity is measured for both people. If there is a difference in acuity between your ears, offer a possible explanation.

C) Transmission of sound

The partner holds a tuning fork approximately 1 m from the subject's right ear. Have the partner strike the tuning fork and the subject make a mental note of the loudness of the sound. Hold one end of a metre stick against your ear and have your partner strike the tuning fork and immediately hold the handle of the tuning fork against the other end of the metre stick. Notice the difference in the intensity of the sound.

The partner strikes the tuning fork and places the handle on top of the subject's head. Notice where the sound appears to come from. Repeat the procedure but place one hand over one ear. Does this affect where the sound appears to be

PROCEDURE: Continued

coming from? Next cover both ears with your hands and have your partner place the vibrating tuning fork over the temporal bone and the mastoid process. Which position gives a better reception of sound waves? How are these sound waves carried to the cochlea?

In a quiet room listen to the ticking of a watch. Have your partner move it towards your ear until the sound is heard distinctly. Measure this distance.

Place the watch between your teeth. Does there seem to be any difference in the loudness of the sound? If so, explain the difference.

D) Equilibrium

Work in groups of three or more for the following exercises. Have the subject sit upright in a swivel chair with his eyes open. The observer stations himself in front of the subject and as close as possible. The other members of the group position themselves around the subject. Rotate the chair clockwise 10 times in approximately 30 seconds.

Observe the movement of the subject's eyes during rotation of the chair as well as immediately upon completion of the 10 rotations. Describe the eye movements associated with nystagmus during rotation. Sketch the movements. Describe the nystagmus movements after the rotations have been completed. Sketch the movements. Note - this happens very quickly, therefore watch the subject's eyes closely.

PROCEDURE: Continued

The same procedure should be repeated but this time the subject tilts his head forward at a 30^{o} angle. Describe and sketch the eye movements during rotation and immediately after the completion of the rotation.

Use a different subject and repeat the procedure. Have the new subject place his head on one shoulder or on his knees during the rotations. Note the eye movements upon completion of the rotations.

Repeat the procedure with a new subject and have him sit in an upright position with his eyes open. Upon completion of the rotation the subject attempts to walk a straight line, approximately 3 m in length. Sketch the path the subject actually walked.

Position a waste paper basket in front and about 2 m away from the subject. Repeat the procedure with the subject in an upright position. At the completion of the rotations have the subject throw a piece of crumpled paper into the waste basket. Describe the results. Which way does the subject throw the paper in relation to the rotation of the chair?

QUESTIONS:

1. What affect does water have on the transmission of sound waves?
2. What principle is applied in the use of a hearing aid?
3. What is the function of the Eustachian tube?
4. What is the cause of motion sickness?
5. If you close your eyes and tilt your head, you know what position your head is in. What organ is responsible for this?

INVESTIGATION 43

EPIDERMAL RECEPTORS

INTRODUCTION:

Sensations of the skin are the cutaneous sensations and they include, touch, pressure, heat, cold and some types of pain.

PURPOSE: To determine the presence and the relative numbers of hot, cold and pressure receptors in the skin.

MATERIALS: dividers (from a geometry set)

nichrome wire, copper wire or any wire of 22-26 gauge

grease pencil

metric ruler

nails

beakers

Bunsen burner or hot plate

ice

tongs or forceps

ring stand and ring

PROCEDURE: A) Pressure discrimination

Working in pairs, the partner should draw a square, 3 cm on a side, on the inside surface of the subject's forearm. A similar square is also drawn in the subject's notebook. Using a piece of nichrome wire, the partner probes the skin within the square on the subject's forearm. The pressure applied should be constant. Wait about 5 seconds between administrations. Record the subject's response to the probing on

PROCEDURE: Continued

the square in the notebook. Note - at least 20 trials should be made. Reverse the roles and repeat the procedure in order that data is obtained for both partners. Explain your results.

B) Temperature discrimination

Place 6 nails in hot water (80°C-100°C) and another 6 in ice water. Draw a 3 cm square on the subject's palm as well as on the back of his hand with a grease pencil. Subdivide the larger square into 16 smaller squares. Draw 2 similar squares in the subject's notebook and label them appropriately.

With the subject's hand resting on the table, the partner should explore each of the 16 squares with either a hot or cold nail. Randomly choose a nail and shake it to remove any excess moisture. Touch the skin within one of the squares and record the subject's response in the appropriate square in his notebook. Also note whether his response is correct or incorrect.

A fresh nail should be used for each test. Peeking by the subject is not allowed!

Repeat the procedure for the opposite side of the hand.

The subject and partner should exchange roles and repeat the procedure.

C) Touch receptors of the skin (Two-point threshold)

Have the subject sit at a table with his arm resting on the table with the palm facing upward. Set a pair of dividers approximately 2 mm apart. Touch the tip of the subject's index finger with the dividers and ask him how many points he feels. If he says "two", reduce the space on the dividers

PROCEDURE: Continued

slightly and repeat the procedure. Keep adjusting the dividers until the subject feels one point. At this time the partner starts to spread the dividers slightly until the subject detects 2 points again. Record this distance in mm, it represents the two-point threshold of the finger tip.

Repeat the exercise and determine the two-point threshold for the back of the hand, the palm, inside of the forearm, back of the neck, sole of the foot, cheek, back and thigh. Record the thresholds in the form of a table.

Reverse the roles of partner and subject and repeat the procedure.

Explain the results of the experiment.

QUESTIONS:

1. What types of skin receptors seem to be most numerous?
2. In what part of the body are touch receptors most numerous?
3. Why are there different numbers of receptors in different parts of the body?
4. Which type of receptors are more common: hot or cold?
5. Why do you sometimes feel chilled when you step into a warm or hot shower?

INVESTIGATION 44

SENSE OF SMELL

INTRODUCTION:

The receptors for the sense of smell are located in the olfactory epitheliu lining of the nasal passages. Classification of odors appears to depend up on the shape of the molecule and it is greatly subjective. Seven basic odors are thought to exist, these being putrid, pepperminty, pungent, fragrant, musky, etheral and camphoraceous.

PURPOSE: To observe some characteristics of the sense of smell and to show the relationship between smell and taste.

MATERIALS: putrid material (meat, bread or beans that have been kept damp for 1 week)

perfume or cologne

vinegar

burned paper or wood

tincture of camphor

oil of peppermint

oil of wintergreen

musk perfume

pieces of apple, raw onion, raw potato

distilled water

PROCEDURE: A) Olfactory receptors

Work in pairs for the following exercises. The subject should not look at the samples as they are used.

PROCEDURE: Continued

Have the subject close his eyes and then the partner holds a number of solutions under the subject's nose. The subject should try to identify each substance by odor. Use the following samples: a) perfume, b) vinegar, c) burned material, d) putrid material, e) camphor, f) oil of peppermint, g) musk perfurme and h) distilled water.

Record the results in a suitable chart. Indicate correct and incorrect responses. Note - wait at least 5 seconds between tests.

B) Adaptation of the sense of smell

Using the index finger of one hand press on the side of one nostril until it is firmly closed. Smell the tincture of camphor in the second nostril until the odor can no longer be detected. Immediately the subject should attempt to distinguish between oil of peppermint and oil of wintergreen. Note the results. How do the results obtained indicate the adaptation of the sense of smell?

C) Retention of odor

The subject should hold one nostril closed and smell the sample of perfume. Breathe out through the mouth and record how long it takes before there is a marked decrease or disappearance of the odor. Open the plugged nostril and smell the perfume. Describe the sensation.

PROCEDURE: Continued

D) Smell and taste

Have the subject rinse his mouth and dry his tongue. While keeping his tongue outside his mouth have the subject pinch both nostrils. The partner now places a piece of raw apple on the subject's tongue. Record the taste sensation. Repeat the procedure and use raw onion and raw potato.

Repeat the procedure using raw apple, onion and potato but this time the nostrils are not pinched. Record the results. How has the identification of these foods changed?

The subject and partner should reverse the roles and repeat the procedure.

QUESTIONS:

1. Describe the location and structure of smell receptors.
2. What is the relationship between taste and smell?
3. What happens to the sense of taste when a person has a bad cold? Explain.
4. To what portions of the brain are olfactory sensations channelled?

INVESTIGATION 45

TASTE RECEPTORS

INTRODUCTION:

The receptors of taste are the taste buds located on the tongue. The neurons of the taste buds are rod-like in appearance. They have "taste hairs" on their outer ends and they represent dendrites, the sensory end of a neuron. The materials to be tasted must fit on receptor sites on the taste hairs. This ensures that the neuron is depolarized and this causes an impulse to be sent to the appropriate area of the brain. The four basic taste sensations that can occur are; sour, salty, sweet and bitter.

PURPOSE: To determine the location of various taste receptors for the basic taste sensations.

MATERIALS: 10% sucrose solution

10% NaCl solution

0.1% quinine sulphate solution (aspirin solution may be substituted)

1.0% acetic acid solution

crystalline sucrose

crystalline NaCl

small pieces of lemon

small pieces of aspirin

toothpicks

cotton

beakers

microscope

prepared slide of taste buds

hand lens

PROCEDURE: A) External structure

With the aid of a hand lens observe your partner's tongue. Locate an area where there is a large concentration of papilla. Describe the physical appearance of the papillae. Note the size of the observed papillae. Are some papillae larger than others? If so, in what portion of the tongue are they the largest? Sketch a top view of the tongue indicating the location of the papillae. Where are the taste buds located?

B) Internal structure

Study a prepared slide of a taste bud and note the appearance of the anterior surface of the tongue. Sketch, under high power, a view of two papillae and the depression located between them. Label each of the following, taste pores, taste bud composed of neurons and papillae. Note the position of the taste buds and their distribution on the papillae.

C) Taste receptors

For this exercise, the subject must rinse and dry his tongue after each solution. Note - chewing gum, candy, or other food will invalidate the results.

Label four clean beakers with acetic acid, quinine, sucrose and salt. Place 10 ml of the solution into the appropriate beaker. Roll a small swab of absorbent cotton on one end of a toothpick and place one of these applicators into each beaker.

In your notebook draw four diagrams of the tongue and label them as follows: sweet, sour, salty and bitter. The subject should now rinse his mouth with fresh water, stick out his tongue and

PROCEDURE: Continued

dry it thoroughly with a piece of clean paper towelling. The subject is to leave his tongue outside his mouth.

The partner should now apply one of the solutions to a particular area of the tongue. Only a single touch of the applicator is required. Replace the swab in the appropriate beaker. The subject now identifies the taste as being sweet, sour, salty or bitter without taking his tongue back into his mouth. This is extremely important. Explain what happens if the tongue is brought back into the mouth. The subject should respond to each application within 5 seconds. The partner keeps track of the response and location of the application on the appropriate diagram. By using a set of symbols record whether the sensation is strong, mild or not noticed. This process is called mapping the tongue. Test the tip, edges and back of the tongue for all four solutions. A minimum of 15 applications should be used for each solution to achieve accurate results.

The subject should now rinse his mouth and then dry it on a clean paper towel. Repeat the procedure for the remaining solutions. The roles of partner and subject should be reversed in order that data is collected for both individuals. Explain why certain tastes were noticed in certain areas of the tongue.

PROCEDURE: Continued

D) Taste and state of substances.

The subject's tongue must be completely dry for this portion of the experiment. Place a small amount of crystalline sucrose on the subject's tongue, after a few seconds, with the tongue still outside the mouth the subject should attempt to identify the substance. Repeat the procedure with crystalline NaCl, small pieces of aspirin and a piece of lemon. Record the results in a suitable chart and explain your results. The subject and partner should reverse roles and repeat the procedure.

QUESTIONS:

1. Are the four types of taste bud receptors located in specific areas of the tongue? Sketch an anterior view of the tongue indicating the four areas.
2. Which substances caused long lasting sensations?
3. In what state do the substances have to be to be detected? Explain why this is the case.

INVESTIGATION 46

THE EYE

INTRODUCTION:

The eyes are photoreceptors which are specialized to respond to light energy. They are enclosed within the orbits of the skull and their movements are controlled by six extrinsic muscles. The orbits protect the majority of the eye while the anterior portion is protected by the conjunctiva and the eyelids.

PURPOSE: To study the structure of the eye.

MATERIALS: sheep or cow eye (fresh or preserved)
dissecting tray
pins
scalpel
scissors
forceps
probes
color chart of the eye
model of the eye

PROCEDURE: A) External structure

Obtain an eye and place it in a dissecting tray. Note the conjunctiva which is represented by a thin membrane lining the eyelids as well as covering the anterior portion of the eyeball. Notice the amount of the eyeball that is covered by the conjunctiva. Are there any portions of the six extrinsic muscles on the surface of the eyeball? Use the

PROCEDURE: Continued

model of the eye or a chart to draw a frontal view of the eye and label the six extrinsic muscles. Notice the fat that is attached to the eyeball. What is the function of this fat?

The sclera (scleroid coat) is the tough, white outer membran of the eyeball and it is continuous with the transperant cornea. When you look at the white of a person's eye, what are you looking at? The optic nerve is seen at the posterio part of the eye. Attempt to locate the stump of the optic nerve. Note the position of this nerve in relation to the eye. Is it in the center of the eye?

B) Internal structure

Using a sharp scalpel and a pair of scissors make a frontal section of the eye. The cut should be made approximately halfway between the anterior and posterior of the eye. Carefully place the anterior portion in such a position that the fluids do not leak out.

Observe the posterior portion of the eye and note the large cavity filled with a fluid called the vitreous humor. Describe its color and viscosity. Carefully remove the vitreous humor and note the innermost layer of tissue. It may be shrivelled and bunched up at one point. This tissue is the retina, the functional part of the eye. What color does it appear to be in the preserved eye? Observe the retina in the model and note the color. Explain the

PROCEDURE: Continued

difference. What color would you expect it to be in a living eye?

Look at the inside of the eye where the optic nerve exits. Remove the retina and note the depression called the optic disk. What seems to happen to the layers of the eye tissue at this point? Why is this known as the blind spot? The layer of tissue immediately next to the retina is the pigmented choroid coat. Use a pair of forceps to lift away some of this layer from the outer sclera. Notice the choroid coat is blue-black in color. Of the three layers of tissue, which one contributes most to the shape and protection of the eye?

Using the anterior portion of the eye drain any remaining vitreous humor from the cavity and note how far the retina extends anteriorly. Remove any remaining retinal tissue. The raised portion of the choroid coat which attaches to the lens is the ciliary body. It controls the shape of the lens as well as the iris. The iris constitutes the colored portion of the eye and the light admitting hole in the choroid coat is called the pupil. Suspensory ligaments anchor the lens to the ciliary body. From what portion of the lens are the suspensory ligaments radiating?

Carefully remove the lens and sketch a frontal view and a side view of it. Notice it is quite hard and rubbery. Why does the lens take a convex shape? Immediately anterior

PROCEDURE: Continued

to the lens is a ring of tissue. What name is given to the tissue and the hole that is found in the center? Observe the fluid that is found in this area of the eye. Compare the color and viscosity of this aqueous humor to the vitreous humor found posteriorly to the lens. The vitreous humor was found in the posterior chamber while the aqueous humor is located in the anterior chamber which is located between the cornea and the iris.

Sketch a sagittal view of the eye and label the conjunctiva, aqueous humor, anterior chamber, cornea, iris, lens, ciliary body, suspensory ligaments, posterior chamber, vitreous humor, optic nerve, optic disk, fovea, retina, choroid coat and sclera.

QUESTIONS:

1. Why do a person's eyes appear to be sunken after a long illness?
2. What type of nerve is the optic nerve?
3. What purpose does the choroid coat serve?
4. Why do some animals' eyes shine in the dark?
5. What is a cataract?
6. What controls the shape of the lens?
7. If a person had crossed eyes (strabismus) what would most likely be the cause?

INVESTIGATION 47

THE HUMAN EYE

INTRODUCTION:

The functional part of the eye is represented by the retina, the innermost layer of the eye. It extends anteriorly to the posterior border of the iris. It contains rod and cone cells which represent the visual receptors of the eye. The nerve fibers of the retina form the optic nerves which exit from the eye, passing to the underside of the cerebrum.

PURPOSE: To discover some of the functional aspects of the eye.

MATERIALS: colored yarn (Holmgren test)
10 x 15 cm filing cards
6 x 6 cm red paper
white sheets of paper
red sheet of paper
black jar lid
light with 60 W incandescent bulb
red, blue, green 60 W light bulbs
5 x 5 cm black, white, red, green and blue cards mounted on sticks
grease pencil

PROCEDURE: A) Blind spot

On a sheet of plain paper, use a grease pencil to draw a heavy circle about 1 cm in diameter on the left half of the paper. Approximately 10 cm to the right of the circle draw a cross the same size as the circle.

PROCEDURE: Continued

Cover your left eye with one hand and hold the sheet of paper at arms length, directly in front of your face. Stare at the circle and very slowly move the paper toward you. It is important not to let your eye wander. As you bring the paper closer to you a point should be reached where the cross disappears from view. Describe from a physiological point of view what has happened. As you bring the paper closer to your face what happens to the cross. Turn the paper 180^{o} and repeat the process.

B) Positive and negative afterimages

The length of time required for a stimulus to produce a sensation is very short. However, the sensation may last a great deal longer than the stimulus was applied. An electric spark may last for 1×10^{-7} seconds while the visible image lasts much longer. This image is referred to as a visual afterimage. If the afterimage is the same color as the original stimulus it is called a positive afterimage. If the afterimage appears as a complementary color of the image it is referred to as a negative afterimage.

Sit in a darkened room for at least 2 minutes. Turn on the 60 W incandescent light for 1 or 2 seconds and look directly at the light source. Turn off the light and describe the afterimage. Note whether it is a positive or negative afterimage and the duration of the afterimage.

PROCEDURE: Continued

Repeat the procedure but this time close your eyes after you turn off the light and follow the afterimage. Does the afterimage follow your eye movements? Explain why or why not. Substitute the colored light bulbs for the white light and repeat the procedure. Answer the same questions for the blue, red and green light that you answered for the white light.

In a room which is well lit stare for 30 seconds at a 6 x 6 cm red card. After 30 seconds have elapsed stare at the center of a sheet of white paper. Note - the surfaces of the red and white paper must be brightly illuminated to obtain the best results. Continue staring at the white paper for at least 1 minute and describe the results.

Look again at the red card for 30 seconds and then stare intently at the centre of a sheet of red paper for at least 1 minute. Describe the afterimage that appears.

Focus your eyes on a colored object (black jar lid) for 30 seconds and then look at a white sheet of paper for 1 to 2 seconds. Close your eyes for at least 1 minute and describe the afterimage that appears.

C) Stereoscopic vision

Hold a pen or pencil with the point up at arms length, at eye level and directly in front of you. Look directly in line with the pen or pencil, focus your eyes on an object in the distance. Describe and explain what happens to the

PROCEDURE: Continued

image of the pen or pencil. Focus on the pen or pencil again. Observe the number of images you see and explain why it has changed from your previous observation.

Hold your hands out at arm's length and at eye level. Have your finger tips touching but spread your fingers so that you can look between them. Focus on some object in the distance while looking through the spaces of your fingers. Describe the appearance of your fingers and make a sketch of them. Physiologically describe your observations.

D) Color blindness (Holmgren test)

Obtain an envelope containing paired pieces of colored yarn and have your partner spread these on a flat surface so that identical colored pieces of yarn are not next to one another. The subject then pairs the similar pieces of yarn. Record any incorrect pairings or difficulties encountered. Are you color blind? If so, with respect to which colors.

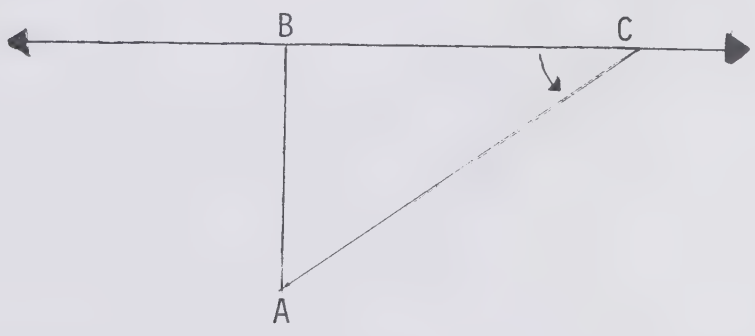
Note - charts for color blindness are readily available and they will give an accurate reading of color blindness. Reverse roles of partner and subject and repeat the procedure.

E) Color sensitivity of the retina

Have the subject sit in a chair facing a blank wall (white is best) and have him focus on a distant point. The subject's eyes and head should be fixed straight ahead at all times. Have the subject cover his left eye with his hand. Hold a colored card on the end of a stick approximately 20 cm directly in front of his right eye. Jiggle the card up and down while

PROCEDURE: Continued

moving the card laterally to the right. The lateral movement should be very slow. Have the subject indicate at what point the color of the card can no longer be distinguished. At this point substitute another card in an attempt to confuse the subject. Record the angle at which the subject loses color sensitivity. Record the data for each colored card. Does the angle remain the same for all colors? What color does the card appear to be when the color is no longer detectable? Repeat the procedure covering the right eye. Explain why the movement of the card causes the color to no longer register on the eye. How does the distribution of rods and cones affect color vision?



Line AB is the line of sight and $\angle$ BCA is the angle of loss of color.

F) Dominant eye

Stand approximately 3 m away from and facing the corner of the room. Focus your eyes on the vertical line of the corner. Extend one arm and hold your thumb extended, in an upward direction. Move your arm until your thumb is in the

PROCEDURE: Continued

line of sight with the corner of the room. Now close your right eye and describe what happens. Next close your left eye and describe what happens. Which eye is dominant? How can you tell? Record your eye dominance and whether you are left or right handed on the blackboard. Do most right handed people have a dominant right eye? What is the ratio of right eye dominant individuals to left eye dominant individuals?

G) Visual acuity test (optional)

Hang a Snellen eye chart at eye level and stand 6.1 m (20 feet) from the chart. Cover your left eye with your hand or a filing card. The partner should stand near the chart and direct the subject to read each line of letters starting at the top of the chart. If the subject is able to clearly read the line marked for vision at 6.1 m (20 feet), he has 20/20 vision. The numerator indicates the distance from the chart, while the denominator indicates the accredited distance he can clearly read the line at. Note - all letters must be read correctly in the line used. For example a person that has 20/30 vision is standing 20 feet (6.1 m) away but can only read the letters clearly which are designated fo a person with normal vision at 30 feet (9.1 m). Repeat the procedure for your left eye, remember to cover the right eye. If the subject wears glasses, the exercise should be done wit and without glasses. If contact lenses are worn, removal of

PROCEDURE: Continued

lenses should be optional.

QUESTIONS:

1. What causes double vision?
2. What happens to the pupil of the eye as the light intensity changes?
3. What is an astigmatism?
4. What is tunnel vision?
5. What is myopia? How can this condition be corrected?

INVESTIGATION 48

REFLEX ARCS

PURPOSE: To study reflexes in humans.

MATERIALS: percussion hammer
glass rod
10 x 15 cm filing cards
flashlight
pencil
20 x 20 cm piece of newspaper
beaker
nail

PROCEDURE: A) Patellar (knee-jerk) reflex

Most reflex actions in humans require at least three neurons to form a complete reflex arc. A sensory (afferent) neuron, an interneuron (internuncial) and a motor (efferent) neuron. The knee-jerk reflex appears to have only two of these neurons, the sensory and the motor neurons. To test this reflex work in pairs.

Have the subject sit on the edge of a table with one leg dangling freely. The subject's partner should locate the knee cap. Once the patella has been located strike the tendon just below it with the percussion hammer. Note and record the results. Sketch a diagram of the reflex arc and label the receptor, sensory neuron, dorsal root ganglion, dorsal root, white matter, cerebrospinal canal, gray matter,

PROCEDURE: Continued

ventral root, motor neuron and effector.

B) Cilio-Spinal reflex

While watching the subject's pupils pinch the skin on the back of the subject's neck. Note and record the results.

C) Uvular reflex

Have the subject open his mouth widely and the partner should gently touch the uvula lightly with a clean glass rod. Note and record the reaction. What function does the uvula have?

D) Pupillary reflex

Have the subject close his eyes for 2 minutes. Hold a 10 x 15 cm filing card along the bridge of the subject's nose so that the left eye is shielded from the right. Shine a bright light into the right eye as soon as the subject opens his eyes. Observe the pupils of both eyes and record the results. Explain your observations fully.

E) Accomodation reflex

Focus both eyes on the tip of a pencil held at eye level and at arms length. While focusing on the pencil tip, describe the objects in the background. Keeping the same line of sight, focus on the objects in the background. What happens to the image of the pencil? Physiologically, what has occurred in this experiment?

PROCEDURE: Continued

The distance from the eye to the nearest object that can be focussed clearly is called the near point of vision. To find the near point shield one eye with a 10 x 15 cm filing card and focus the uncovered eye on the print of a piece of newspaper. Gradually bring the newspaper toward your eye until the letters can no longer be seen clearly. Have your partner measure this distance in centimetres. Repeat this procedure for the other eye and record the data. Is the near point the same for both eyes? Explain your answer.

F) Conditioned reflex

When a new stimulus elicits the same response as the old stimulus, conditioning is said to have occurred. This exercise may require three individuals.

The subject should sit in a chair in a dimly lighted room. One partner flashes a light in the subject's eyes and at the same moment the third member of the group strikes a beaker with a nail to produce a sharp sound. The timing of the light flash and the sound is critical. Repeat this procedure 15 times at 30 second intervals. On the 16th trial, strike the beaker but do not shine the light in the subject's eyes. Watch the pupils of the subject's eyes. Record your observations. If no reaction is observed, repeat the procedure and record the results.

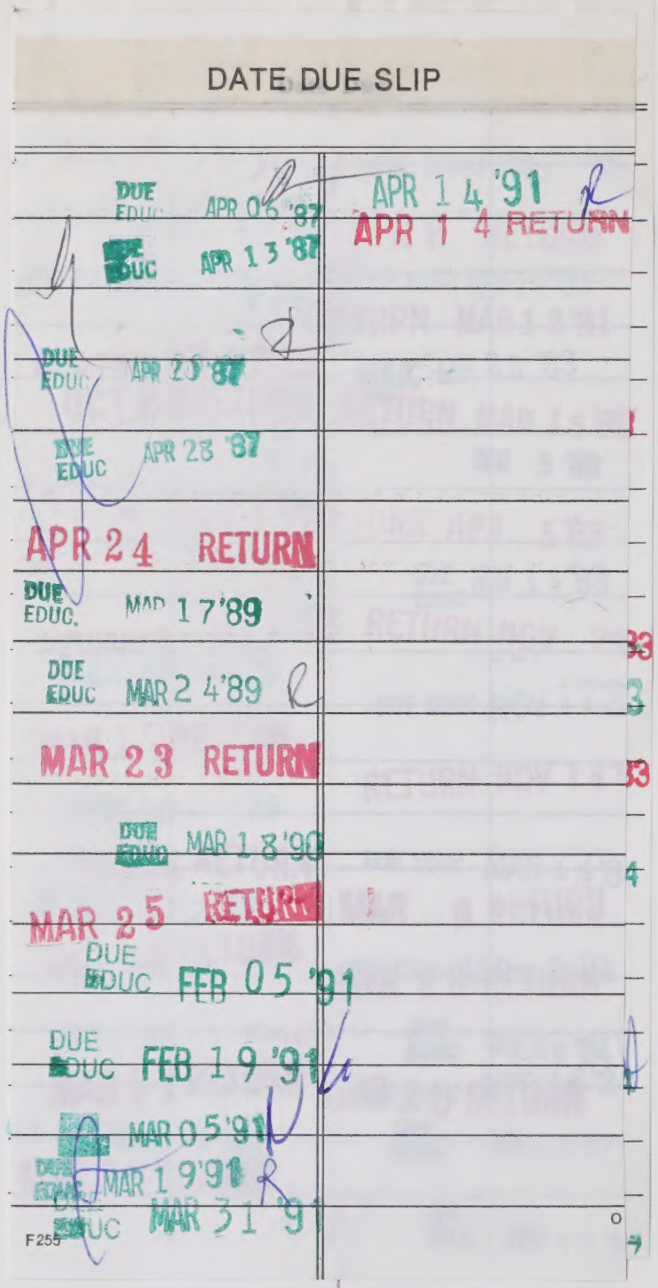
If a good conditioning process occurred, strike the beaker a few times at 10 second intervals and watch what happens

PROCEDURE: Continued

to the pupillary reflex. Record your results.

QUESTIONS:

1. What are some benefits of conditioned reflexes?
2. What could possibly happen if the pupillary reflex did not exist?
3. What physical structure controls the size of the pupil?
4. Why can a person not see objects clearly when they are positioned closer to the eye than the near point?
5. What happens to the near point as a person gets older? Why does this occur?
6. How can the condition in question 5 be corrected?



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